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PLANT CELL BIOLOGY AND DEVELOPMENT 14

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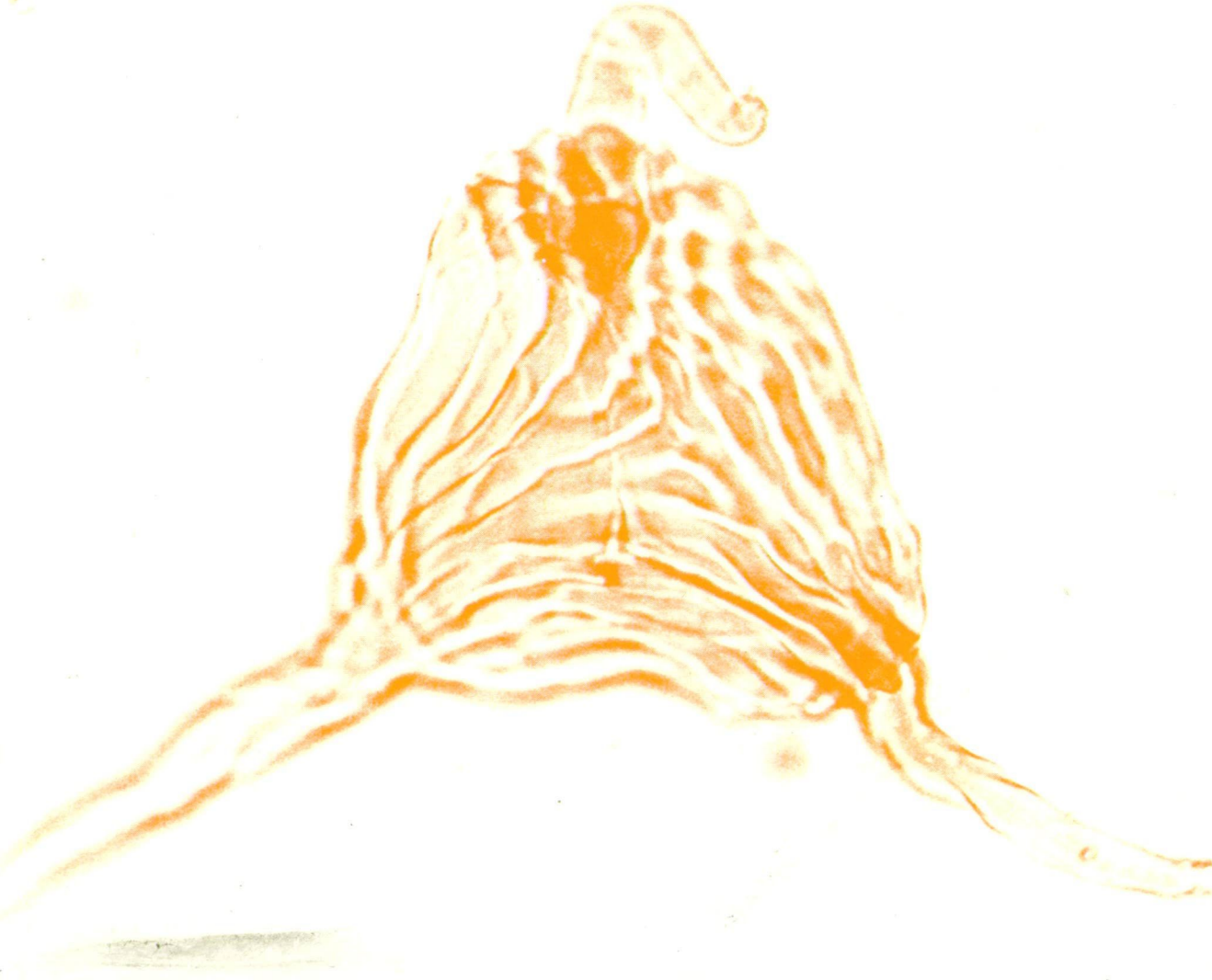

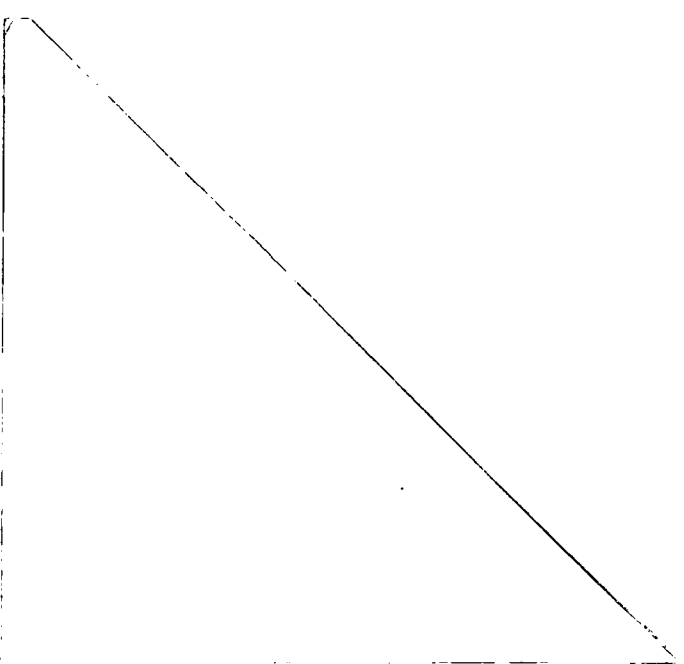

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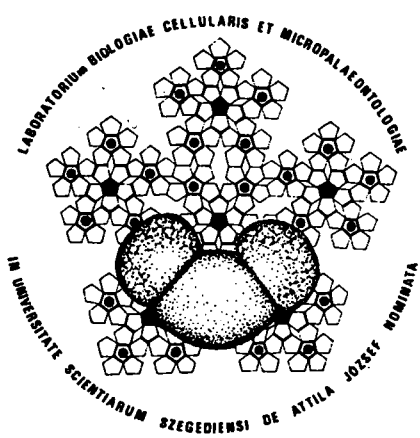

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Preface

After the new Millennium, and the 10th Anniversary of the Laboratory we started the normal life of our group. The international joint research programs broadened out, and hopefully it will not change in the future. The activity of the young members of the Laboratory is unchanged on the high level.

During the last year we lost Prof. Dr. K. BURGER, member of the Hungarian Academy of Sciences, who was always the aid of our Laboratory in his different positions, scientific rector adjoint, president of the Regional Committee of the Hungarian Academy of Sciences. Dr. R. ZÁNTHÓ associated professor deceased during the last year. He was helping in the linguistic corrections of our manuscripts written in English.

On the 3th April was the 30th Anniversary of the foundation of the Biological Reseach Center of the Hungarian Academy of Sciences. The Electron Microscope Laboratory of the Department of Biophysics has been very important for our scientific research programs from the beginning, because it has made possible to carry out ultrastructure studies in this Laboratory that are in every respect very useful not only of the researches, but in the special teaching of the Laboratory.

At the 11th Anniversary of the Laboratory, on the 21th August an exclusive reception was held. At this occasion the following personalities were awarded with the Commemorative Medal of the Laboratory:

ERIC CAULTON, Director, Scottish Centre for Pollen Studies, Edinburgh, Scotland, UK.,

Dra. Maria-Teresa FERNÁNDEZ MARRÓN, Departamento de Paleontologia, Facultad de Ciencias Geológicas, Universidad Complutense de Madrid, Madrid, Spain.

Dr. S. K. M. TRIPATHI, Birbal Sahni Institute of Palaeobotany, Lucknow, Uttar Pradesh, India, was awarded with the Millennium Medal of the Laboratory.

For the generous financial support of the publication of this number I would like to express my sincerest thanks:

to the Grant OTKA T 31715,

to the Grant A.K.P. PFP 1300-54,

to the Foundation for Szeged,

to Prof. Dr. R. MÉSZÁROS, Rector of the University of Szeged.

It is regrettable that the Faculty of Science of the University of Szeged and the Regional Committee of the Hungarian Academy of Szeged were not able to contribute to the printing costs of the publication of the Laboratory. Another disadvantegeous event that the Department of Botany cannot assure the financial support to the function of the Laboratory which was established in the statutes of the Laboratory (No. 25-B-1990) by the Rector of the University.

Szeged, 30. December. 2001.

M. KEDVES
Head of the Laboratory

SOBRE LA ENTREGA DE LA MEDALLA DEL MILENIO CON MOTIVO DE HABERSE CUMPLIDO EL DÉCIMO ANIVERSARIO DE LA CREACIÓN DEL CELL BIOLOGICAL AND EVOLUTIONARY MICROPALEONTOLOGICAL LABORATORY

Tengo el agrado de dirigirme al Prof. Miklós KEDVES con el objeto de agradecerle profundamente el haber sido distinguida con la Medalla del Milenio del Laboratorio que el Prof. conduce con motivo de haberse conmemorado los 10 años de su creación.

Esta distinción en reconocimiento a mi producción científica dentro del campo de la Palinología constituye para mi un alto honor ya que tal nominación proviene de un grupo de trabajo de larga, prolífica y reconocida trayectoria en la especialidad. Las contribuciones de ese Laboratorio forman parte de nuestras bibliotecas y son fuente de consulta permanente tanto para enseñanza como para investigación. Tengo además, desde hace unos años, el honor de integrar el Comité de Árbitros de la prestigiosa publicación que produce.

Creo propicia esta oportunidad para destacar a quienes hicieron posible el mejoramiento de mis conocimientos elevándolos al nivel que hoy merece esta mención. En primer lugar mi familia y amigos quienes con su respeto, comprensión y apoyo permanente me brindaron la energía y la paz necesaria para poderme dedicar de lleno a estos objetivos. A mis maestros de la Facultad de Ciencias Naturales y Museo de La Plata. A la Universidad Nacional de La Plata y Consejo Nacional de Investigaciones Científicas y Técnicas, instituciones de mi país que subvencionan y apoyan nuestro trabajo de investigación. Resultaron decisivas en los últimos doce años las oportunidades brindadas por instituciones de mi país y extranjeras que me permitieron contactar y posteriormente trabajar junto a personalidades descollantes en la especialidad en países tales como Japón, Inglaterra y Suecia. Gracias a las becas otorgadas por el Instituto Sueco he podido desarrollar mi trabajo en el Departamento de Botánica de la Universidad de Estocolmo en colaboración con el Dr. John R. ROWLEY, lo cual me posibilitó incorporar nuevas técnicas y metodologías y utilizar el más moderno y sofisticado instrumental de alta resolución. Gracias a la generosidad de los maestros y colegas de todos los países visitados he podido acceder al saber acumulado por los mismos y apreciar las distintas perspectivas que se pueden abordar lo que posteriormente me permitió desarrollar nuevas líneas de trabajo en mi país. Toda esta experiencia me condicionó a cambiar la óptica sobre la disciplina en general y a la vez modificar las perspectivas de la especialidad en mi país en lo que a mi esfera de acción se refiere. Finalmente una mención especial va para mis colegas y discípulos por elegirme y dedicar su tiempo e interés para llevar adelante estos proyectos, profundizarlos y enriquecerlos con sus aportes personales.

Por las razones enumeradas y con motivo del honor conferido a mi persona por el Cell Biological and Evolutionary Micropaleontological Laboratory comprometo mi conocimiento y esfuerzo en lograr nuevos frutos para contribuir al conocimiento de los mecanismos biológicos fundamentales a los cuales podemos acceder a través del desarrollo de esta disciplina.

Gracias pues una vez más por el reconocimiento, la hermosa medalla recibida será exhibida permanentemente con orgullo en nuestro laboratorio.

Dra. Marta A. MORBELLI

1. TYPES OF SPOROMORPHS FROM THE AJKAITE CONTAINING BROWN COAL SAMPLES FROM AJKA (HUNGARY)

TIPOS DE ESPOROMORFOS DETERMINADOS EN MUESTRAS DE LIGNITO DE LAS MINAS DE AJKA (HUNGRIA)

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Abstract

This paper present the quantitative data of the spore pollen types of the two amber-containing brown coal samples from among which the Ajkaite material is under transmission electron microscope investigations. The two samples are from the same level of the brown coal layers, but the composition of the sporomorphs is completely different. The brown coal forming vegetation based on the palynological data was not the same in the different part of this kind of sedimentary basin. It may be supposed, that the "amber tree" of the Ajkaite during the Upper Cretaceous period in the brown coal basin of Ajka, Hungary was not always the same.

Key words: Palynology, Upper Cretaceous types, Ajkaite containing brown coal.

Resumen

Se dan a conocer los datos cuantitativos de tipos de esporas y pólenes hallados en las capas de lignito del Cretácico Superior de las Minas de Ajka, donde se encuentra el ámbar (Ajkaite) que está siendo investigado al Microscopio Electrónico de Transmisión.

Las dos muestras analizadas proceden de un mismo nivel de lignitos, aunque los porcentajes de esporomorfos son diferentes.

La vegetación que originó el carbón que contenía las muestras de ámbar no era uniforme como se desprende de los datos palinológicos, lo que hace suponer que los árboles que originaron el ámbar en los lignitos estudiados, no formaban un bosque homogeneo.

Palabras clave: Palinología, Cretácico superior, lignitos de Ajka (Hungría).

Introduction

In our previous papers (KEDVES, SZÓNOKY, MADARÁSZ and KOVÁCS, 2000, KEDVES, BORBOLA and PRISKIN, 2001) the first results of the new programme of our Laboratory were published. After beginning, the most important result was that the ultrathin sectioning of the Ajkaite was succesful. To obtain informations on the origin of the "amber tree", the fragments of the secondary xylem remnants were investigated qualitatively and quantitatively. Important differences were established between the two

samples with this method. Following this kind of LM studies, detailed spore-pollen analyses were carried out. LM pictures were taken from all microfossils from 25 slides per samples. Taking into consideration the problems in the taxonomy of the Upper Cretaceous sporomorphs, the detailed taxonomy will be elaborated by groups and will be published later.

The aim of this contribution is, present the quantitative data of the sporomorphs, to have a general overview concerning the spore-pollen composition of the two samples in comparison with the data of the secondary woody fragments.

Materials and Methods

The material for our investigations was published previously in our first paper (KEDVES, SZÓNOKY, MADARÁSZ and KOVÁCS, 2000) the method of the types of the sporomorphs for characterizing the sediments, introduced by KRUTZSCH (1957/58) was used. The advantages of this method were backed up later during the study of the spore-pollen assemblages of the Lower Tertiary layers of Europe, and to the Paris Basin (KEDVES, 1967,1968).

Results

The quantitative data of the two samples investigated are presented in fig. 1.1. Within the represented groups the following types were observed: 1. Spores - Schizaeaceae *Lygodium, Anemia* type, Gleicheniaceae, (laevigate, toriate forms), Pteridaceae, Lycopodiaceae, Selaginellaceae (echinate microspores). 2. Gymnosperm pollen grains - as new data the hiatus group of the Taxodiaceae-Cupressaceae may be emphasized. 3. Monosulcates represents also different types, some of them represents the Arecales (*tranquillus* type, *Retimonosulcites*) but the Cycadales may also be presumed. Interesting further forms with reticulate sculpture are also present in this material. Araceae may be presumed with a striate monosulcate form. 4. Tricolpates are represented with *liblarensis* and further more or less smooth pollen grains and one probably African-Australian group of *Dettmannaepollenites/Phimopollenites* (KEDVES, 1999). 5. Tricolporates represents in the first place of the small Fagaceae types of *Pasania, Castanea* types. There are further types of Longaxones reticulate of polycolpates. 6. Pronormapolles is represented with the *Complexiopollis* form-species. 7. *Hungaropollis* described first by GÓCZÁN (1964) is a very particular Eunormapolles type of the Carpathian Basin with a restricted geographical and time-table distribution. 8. "Oculata Normapolles", mostly of the *Oculopollis* PFLUG 1953 fgen., but further form-genera described in the monograph of GÓCZÁN, GROOT, KRUTZSCH and PACLTOVÁ (1967). 9. Further Eunormapolles, such as *Interporopollenites, Vacuopollis, Suemegipollis, Trudopollis.* 10. "*Momipites* group", small triaperturate pollen grains. Prof. Dr. S. NILSSON was kindly wrote to us his opinion of this kind of fossil pollen grains as follows: "Regarding the pollen grains I immediately got the family Myrtaceae in my mind. However, similar grains also occur in the Sapindaceae or, less probably, the Loranthaceae. So Myrtaceae is my first guess". 11. Further Postnormapolles (PFLUG. 1953), triporate (probably early type of the Betulaceae), subtriporate, caryoide forms, polyporate types. 12. Incertae - the damaged forms and the peculiar form of *Pseudoschizaea* (*Concentricystes*) was classed here.

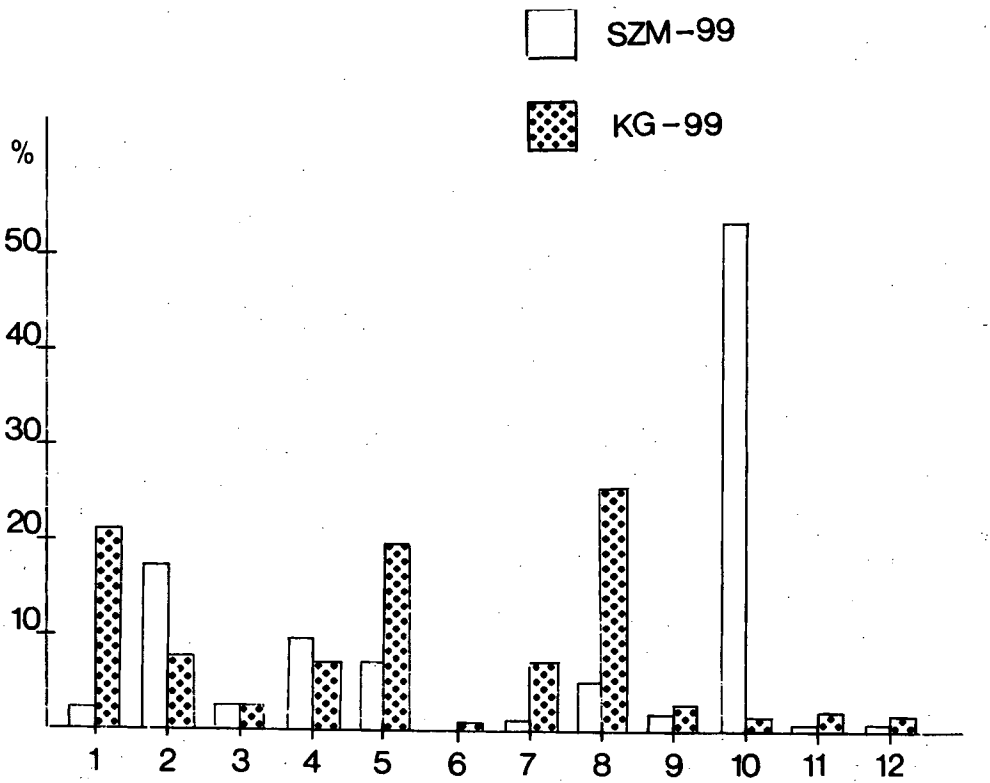

Fig.1.1.

The percentages of the different spore and pollen types from the two Ajkaite containing brown coal samples from Ajka. 1. Spores, 2. Gymnosperm pollen grain, 3. Monosulcates, 4. Tricolpates, 5. Tricolporates, 6. Pronormapolles, 7. *Hungaropollis* fspp., 8. Oculata group, 9. Different taxa of further Eunormapolles, 10. Momipites group, 11. Postnormapolles fgen. et fspp., 12. Incertae.

As regards the distribution of the different types of the two investigated samples we can establish the following:

Sample: SZM-99. - Extremely high quantity of the *Momipites* group, with gymnosperms and Longaxones types (tricolpate and tricolporate). The quantity of the Normapolles is relatively low.

Sample: KG-99. - Pteridophyte spores, Longaxones mostly tricolporates and the "Oculata Normapolles" are in a high, more or less in equal quantity. In contrast to the previous sample, the *Momipites* group is infrequent.

Discussion and Conclusions

1. The brown coal-forming vegetation in the two samples investigated was different. In this way it is presumed, that the origin of the amber (Ajkaite) was also not the same.

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2. The extremely high quantity of the *Momipites* group in the sample SZM-99 together with the high quantity of the „Type L" woody remnant („Resinous remnant with the pattern of scalariform vessel perforation" cf. KEDVES, BORBOLA and PRISKIN, 2001, p. 26) is important. An attempt was made to establish the presumed botanical affinity of this kind of vessel, and its evolutionary significance.

3. During our further studies the detailed taxonomy and the botanical affinities will be elaborated.

Acknowledgements

The writers are very thankful for Prof. Dr. S. NILSSON (Palynological Laboratory, Swedish Museum of Natural History, Stockholm, Sweden), and to E. CAULTON (Scottish Centre for Pollen Studies, Edinburgh, Scotland, U.K.) for critically reading the manuscript. This work was supported by Grant OTKA T 31715.

References

GÓCZÁN, F. (1964): Stratigraphic Palynology of the Hungarian Upper Cretaceous. - Acta Geologica *8*, 229-264.

GÓCZÁN, F., GROOT, J.J., KRUTZSCH, W. und PACLTOVÁ, B. (1967): Die Gattungen des "Stemma Normapolles PFLUG 1953b" (Angiospermae) Neubeschreibung und Revision europäischer Formern (Oberkreide bis Eozän). - Paläont. Abh. *B, 2*, 427-633.

KEDVES, M. (1967): Sur quelques problèmes de stratigraphie palynologique appliquée au Tertiaire inférieur en Europe. - Pollen et Spores *9*, 321-334.

KEDVES, M. (1968): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne. II. Tableau de quelques espèces et types de sporomorphes. - Pollen et Spores *10*, 117-128.

KEDVES, M. (1999): Upper Cretaceous pollen grains from Egypt. III. - Plant Cell Biology and Development (Szeged) *10*, 14-29.

KEDVES, M., BORBOLA, A. and PRISKIN, K. (2001): LM and TEM investigations on the Upper Cretaceous Ajkaite of Hungary II. - Plant Cell Biology and Development (Szeged) *13*, 22-31.

KEDVES, M., SZÓNOKY, M., MADARÁSZ, M. and KOVÁCS, G. (2000): LM and TEM investigations on the Upper Cretaceous Ajkaite of Hungary I. - Plant Cell Biology and Development (Szeged) *12*, 8-17.

KRUTZSCH, W. (1957/58): Sporen- und Pollengruppen aus der Oberkreide und dem Tertiär Mitteleuropas und ihre stratigraphische Verteilung. - Zeitschrift für angewandte Geologie *11/12*, 509-548.

NILSSON, S. (2000): Letters communication.

PFLUG, H.D. (1953): Zur Entstehung und Entwicklung des angiospermiden Pollens in der Erdgeschichte. - Palaeontographica *B, 95*, 60-171.

2. LM AND TEM INVESTIGATIONS ON THE UPPER CRETACEOUS AJKAITE OF HUNGARY III.

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Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, H-6701, P.O. Box 993, Szeged, Hungary

Abstract

Light and transmission electron-microscope investigations were implemented on some trilete spores characteristic of the amber layers of the Ajka Brown Coal Basin. The presumed functions of the appendices of the *Appendicisporites tricuspidatus* spore type, with it's restricted regional and stratigraphic occurrences, are discussed in comparison with the elater bearing fossil and extant spores of the genus *Equisetum*. The results of the TEM Ajkaite trilete spore studies are compared with those LM data of the "adriennis" type spore massula.

Key words: Upper Cretaceous, Ajkaite, spores, LM, TEM.

Introduction

The Upper Cretaceous Carpathian Basin spore assemblages have several local or regional restricted elements (KEDVES and DINIZ,1983). Some of these data have been previously published (KEDVES, SZÓNOKY, MADARÁSZ and KOVÁCS, 2000), in particular the Eunormapolles type *Hungaropollis* GÓCZÁN 1964a; and the Schizaeaceous fern spore with long appendices, described by WEYLAND and GREIFELD (1953). The presumed function of the appendices of this type of spore and its stratigraphical and geographical distributions are the subject of this LM study. In addition, the ultrathin sections from the amber have produced the first results concerning the fossil sporoderm.

The aim of this paper is the following:

1. Investigate the spores of "tricuspidatus type" as one of a more or less local element of the European Normapolles Province, together with other local elements, that are found in the Carpathian Basin.

2. Present new ultrastructure TEM data from fossil Schizaeaceae spore type recovered from the Ajkaite.

Materials and Methods

Material from previously published LM studies (KEDVES, BORBOLA and PRISKIN, 2001; KEDVES and ALVAREZ RAMIS, 2002) were used in this investigation. The Ajkaite (sample KG-99) was embedded in Araldite (Durcupan, Fluka). The TEM photographs were taken with the Hungarian Academy of Sciences Department of Biophysics, Biological Research Center's Tesla BS-540 at a resolution of 6-7Å.

Results

LM Results: Nomenclatural question of *Appendicisporites tricuspidatus* WEYLAND and GREIFELD 1953 (Plate 2.1., figs. 1-4)

After the publication of THIERGART (1949) and WEYLAND and GREIFELD (1953) a large number of Lower and Upper Cretaceous spores were assigned to the form-genus *Appendicisporites.* These spores also included a large number that had thickened appendices. Many of these types of spores were placed in the form-genus *Plicatella* MALYAVKINA adding to the nomenclatural confusion. DEÁK (1963, 1965), tried to resolve this taxonomic problem. However, the nomenclature of the fossil Schizaeaceous spores has still not been satisfactorily resolved. For example, DAVIES (1985) in his monograph of the Anemiacean, Schizaeacean and related spores introduced the following taxa: *Plicatella tricuspidata* (WEYLAND and GREIFELD 1953, p. 12, Table 3, fig. 18) comb. nov. Early Senonian: formerly *Appendicisporites, Anemia.* GRANZOW and HELMERICH (1992, p. 439) wrote the following: "1949 wurd von THIERGART "*Sporites appendicifer*" und 1953 von WEYLAND and GREIFELD "*Appendicisporites tricuspidatus*" veröffentlich. Ein gegenüberstellung der beiden Sporen aus dem Senon von Deutschland bzw. Polen ergibt, dass sie eindeutung zur gleichen Formart gehören". These spores were classified as *Plicatella appendicifera.* This present study was restricted to only those spores in which the lengh of the appendices is longer than the spore radius. In regards to the current nomenclatural problems, it is agreed in this paper to accept the concept of WEYLAND and GREIFELD (1953, p. 43) citing the following: "THIERGART bezeichnet die Spore als eine stark spezialisierte kurzlebige Form".

The most important occurrences of the spores of "tricuspidatus type" are as follows:

Alb-Cenomanian. - *A. tricuspidatus* WEYL. and GR., Vojvodina and near Belgrade, Yugoslavia, PANTIC and DULIC (1994).

Cenomanian. - *A. tricuspidatus* WEYL. et GREIF., Pont Bati, Vendée, France, DURAND, TERS and VERGER (1963), *Appendicisporites tricuspidatus* WEYL. et GREIF. 1953, Charente-Maritime, France, DEÁK and COMBAZ (1967), *Anemia tricuspidata* (WEYLAND et KRIEGER) BOLKHOVITINA 1961, Laudun Gard, France, MÉDUS et TRIAT (1969), *A. tricuspidatus* WEYL et GREIF., Peruc Formation, Czech Rep., PACLTOVÁ and SVOBODOVÁ (1992), *A. tricuspidatus* WEYL. et GREIF., Blansko Graben, Czech Rep., SVOBODOVÁ (1997), *Appendicisporites tricuspidatus*, Poganovo Paralic Series Southeast Serbia, PANTIC and DULIC (1999).

Cenomanian/Turonian. - *A. tricuspidatus* WEYLAND et GREIFELD, León, Northern Spain, VAN AMEROM (1965), *A. tricuspidatus* WEYL. et GREIF. Vocontian Basin, Czech Rep., SVOBODOVÁ, MÉON and PACLTOVÁ (1998).

Turonian. - *A. tricuspidatus* WEYL. et GREIF. Sabran, Gard, France, DUCREUX, GAILLARD and SAMUEL (1982).

Plate 2.1.

1-4. *Appendicisporites tricuspidatus* WEYLAND et GREIFELD 1953., slide: KG-99-9, Cross-table number: 22.7/141.2, figs. 1,2. 500x, figs. 3,4, 1.000x.

5. Schizaeaceae spore of "adriennis type"., slide: KG-99-19, Cross-table number: 20.3/140.8.

6. Ultrastructure of a laevigate trilete spore in the amber. Block No.: 99-KG-5, Negative No.: 8103, 5.000x.

7. Detail of the ultrastructure of a massula of laevigate trilete spores. Block No.: 99-KG-5, Negative No.: 8106, 15.000x.

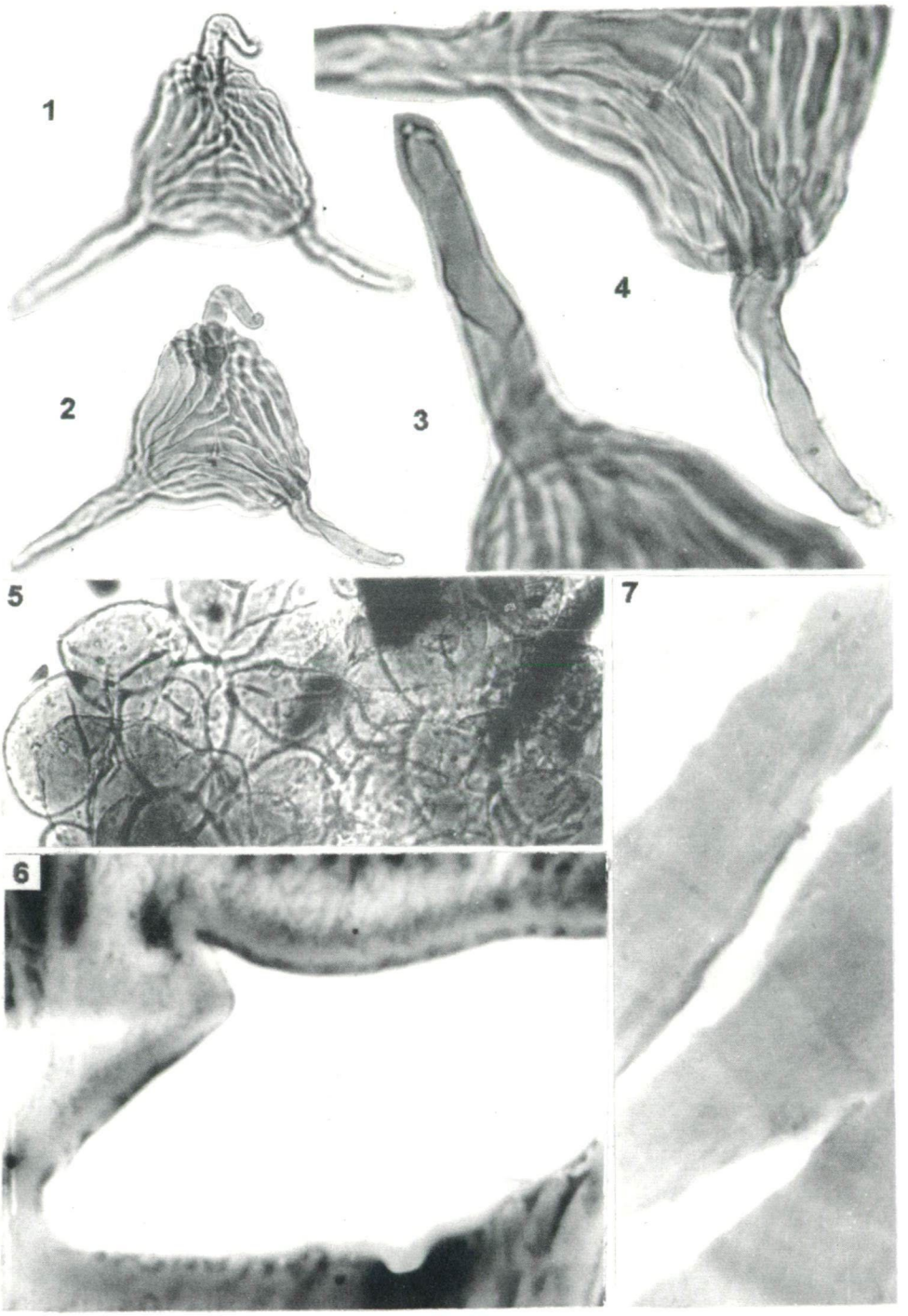

Plate 2.1.

Senonian. - *A. tricuspidatus* WEYL. et GREIF. Quedlinburg Germany, WEYLAND and GREIFELD (1953).

Santonian. - *A. tricuspidatus* WEYL. et GREIF. 1953, Ajka, Hungary, GÓCZÁN (1964a).

Santonian/Campanian. - *A. tricuspidatus* WEYLAND et GREIFELD (15), Bakony, Hungary, GÓCZÁN (1964a,b).

As regards the spores from the brown coal of Ajka we cite from the paper of GÓCZÁN (1964a); p. 233: "3. In zone "C" the spores with verrucate structure (13), *Appendicisporites tricuspidatus* WEYLAND et GREIFELD (15), 40-45 μ sized *Oculopollis* (12), as well as tetracolporate (11) and inaperturate (14) 60 to 100 μ in diameter, are dominant (Figs. 11 to 15)".

Campanian. - Gruppe 3, Pl. I, fig. 21 only, Aachen, Germany, KRUTZSCH (1957). Fulvelian. - *Appendicisporites tricuspidatus*, Saon, France Drome, IPERT (1976).

LM data the of the spores isolated from the brown coal amber bearing layers of Ajka

Amb triangular with convex sides (Plate 2.1., figs. 1,2). The laesurae of the tetrad mark reach the base of the appendices (Plate 2.1., figs. 1,2,4). Sculpture canaliculate, the size of four isolated striae and muri is about 20 μm (Plate 2.1., fig. 4). Diameter of the spore without appendices 48-60 μm, the appendices are 45-55 μm long so the size of the appendices is larger than the radius of the spore. Sometimes the appendices are bended in the direction of the proximal pole (Plate 2.1., figs. 1,2). It may be presumed that in the sporangia, the appendices were close to the spore body as is found in extant spore genus *Equisetum.* The appendices are characteristic twisted well shown in figs. 2,4 in Plate 2.1.

The new TEM results of the spores in the Ajkaite

A massula of laevigate spores of Schizaeaceae of "adriennis type" (Plate 2.1., fig. 5) occurred in our slides. TEM data from similar mass of spores was observed (Plate 2.1., fig. 7). The surface of the exospore is uneven and its inner part is, in general, electron dense. Similar mass of spores from the Paleozoic was published by WELLMAN, EDWARDS and AXE (1998), and WELLMAN (1999). Another spore, without protoplasm, occurred in our ultrathin sections of the amber (Plate, 2.1., fig. 6). The ultrastructure of the laesurae is also well shown with the inner part of the exospore being electron dense.

Discussion and Conclusions

The long appendices of *A. tricuspidatus* may be comparable to the elater bearing fossil and extant *Equisetum* spores. The fossil forms from the Pennsylvanian with three circinately coiled elaters were decribed by WILSON (1943) and assigned to *Elaterites triferens.* There are several papers concerning this subject with the most important ones, in our view, are as follows: POTONIÉ (1956) emphasized the following, p. 56: "Die Gattung zeigt Beziehung zu den mesozoischen Gattung *Appendicisporites* WEYLAND & KRIEGER 1953". GOOD and TAYLOR (1974) published LM and SEM photographs of *E. triferens* spores isolated from *Calamocarpon insignis* microsporangia. Fig. 6, in P. 149, illustrates a twisted elater with a striate surface. GOOD (1975) described several Calamitean cones with associated spores and a wind dissemination was emphasized for the function of the elater in *Equisetum.* "In the case of calamitalen spores it is also possible that elaters functioned as a means of propelling the spores from the sporangium and in doing so became detached" (GOOD, 1975, p. 84). The Mesozoic fossil elater bearing spore - *Equisetosporites* DAUGHERTY 1941 probably has four elaters following the

monograph of POTONIÉ (1956). KEDVES (1979) reviewed the most important basic results of the spores of the extant genus *Equisetum* through LM and SEM investigations of fourteen species. The striate surface of the elaters of these fourteen species was observed and the fine ornamentation was discussed. It is worth of mentioning that there are four elaters in the spores of *Equisetum* in contrast to those of the previously mentioned Paleozoic calamitean spores. After being subjected to high temperatures, the twisting of the elaters is more charactertistic. It can be presumed that the function of the Schizaeaceae appendices is similar to those of the Equisetaceae elaters. It also is noteworthy that the number of these appendices is three and different, in contrast, to the more or less globular Sphenopsida spores that are within the fossil forms of Schizaeaceae. This latter mentioned group, an isolated one of limited stratigraphical and geographical distribution, may have similar morphological features for their dispersion. This type of spore was discussed herein as an addition to the local elèments of the Upper Cretaceous flora of the Carpathian Basin. For example, the genus *Hungaropollis* GÓCZÁN 1964a has a similar restricted distribution. All these data are important for the understanding the fossil vegetation contained in the "amber tree".

The new data presented herein are important for the understanding the ultrastructure of fossil spores. It is hoped that, in spite of the fact that protoplasm was not preserved in the spores of this study, the information will be useful to future investigators. The superficial ultrastructure of the spore mass is comparable to the exospore of *Leiotriletes adriennis* (POTONIÉ et GELLETICH 1933) KRUTZSCH 1959 published first by KEDVES and PÁRDUTZ (1973).

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References

AMEROM, H.W.J. VAN (1965): Upper Cretaceous pollen and spores assemblages from the so-called "Wealden" of the province of Leon (Northern Spain). - Pollen et Spores *7*, 93-133.

BOLKHOVITINA, N.A. (1961): Fossil and present-day spores of the family Schizaeaceae. - Acad. Sci. U.R.S.S., Trud. Geol. Inst. *40*, 1-176.

DAUGHERTY, L.H. (1941): The Upper Triassic flora of Arizona. - Carniegie Inst. Wash., Pub. *526*, 1-108.

DAVIES, E.H. (1985): The Anemiacean, Schizaeacean and Related Spores: An Index to Genera and Species. - Canad. Techn. Rept. of Hydrography and Ocean Sci. *67*, Darthmouth, Nova Scotia.

DEÁK, H.M. (1963): Quelques spores striées de l'étage Aptien. - Revue de Micropaléontologie *5*, 251-236.

DEÁK, H.M. (1965): A Dunántuli Középhegység apti üledékeinek palynológiai vizsgálata. Recherches palynologiques des dépôts Aptiens de la Montagne Centrale de Transdanubie. - Geologica Hungarica ser. Palaeontologica *29-32*, 7-105.

DEÁK, M.H. et COMBAZ, A. (1967): «Microfossiles organiques» du Wealdien et du Cénomanien dans un sondage de Charente-Maritime. - Revue de Micropaléontologie *10*, 69-96.

DUCREUX, J.L., GAILLARD, M.G. et SAMUEL, E. (1982): Un gisement à plantes du Turonien supérieur la carrière de Sabran, à l'ouest de Bagnols sur-Cèze (Gard France) stratigraphie, sédimentologie, étude de macrorestes végétaux et palynologie. - Mém. Mus. Nat. d'Hist. Nat. N.S. C, Sciences de la Terre *49*, 71-79.

DURAND, S., TERS, F. et VERGER, F. (1963): Sur un nouveau gisement d'argile noire cénomanienne dans le marais de Monts (Vendée) et sa signification paléogéographique. - C.R. Acad. Sc. Paris *257*, 1492-1494.

GÓCZÁN, F. (1964a): Stratigraphic Palynology of the Hungarian Upper Cretaceous. - Acta Geologica *8*, 229-264.

GÓCZÁN, F. (1964b): A bakonyi szenon palynológiai standardje. Standard palynologique du Sénonien de la Montagne Bakony. - M.Á.F.I. évi jelentése az 1961. évről *1*, 253-261.

GOOD, C.W. (1975): Pennsylvanian-age Calamitean cones, elater-bearing spores, and associated vegetative organs. - Palaeontographica *B, 153*, 28-99.

GOOD, C.W. and TAYLOR, T.N. (1974): The establishment of *Elaterites triferens* spores in *Calamocarpon insignis* microsporangia. - Trans. Amer. Micros. Soc. *93*, 148-151.

GOOD, C.W. and TAYLOR, T.N. (1975): The morphology and systematic position of Calamitean elater-bearing spores. - Geoscience and Man *11*, 133-139.

GRANZOW, W. und HELMERICH, K.-D. (1992): *Plicatella appendicifera* (Sporomorphae) - eine taxonomische Betrachtung. - Senkenbergiana lethaea *71*, 439-445.

IPERT, C. (1976): Données palynostratigraphiques sur les formations des sables à lignites Dieulefit, Nyons et Saon (Drome). - Géobios *9*, 97-100.

KEDVES, M. (1979): Az *Equisetum* nemzetség recens spóráinak LM és SEM vizsgálata. Testing of the spores in the *Equisetum* genus. - Bot. Közlem. *66*, 195-203.

KEDVES, M. and ALVAREZ RAMIS, C. (2002): Types of sporomorphs from the Ajkaite containing brown coal samples from Ajka (Hungary). Tipos de esporomorfos determinados en mestras de lignito de las minas de Ajka (Hungría). - Plant Cell Biology and Development (Szeged) *14*, 7–10.

KEDVES, M., BORBOLA, A. and PRISKIN, K. (2001): LM and TEM investigations on the Upper Cretaceous Ajkaite of Hungary II. - Plant Cell Biology and Development (Szeged) *13*, 22-31.

KEDVES, M. et DINIZ, F. (1983): Les Normapolles du Crétacé supérieur en Europe: Implications Paléobiogéographiques. - Geobios *16*, 329-345.

KEDVES, M. and PÁRDUTZ, Á. (1973): Ultrastructure examination of fossil Pteridophyta spores and Gymnospermatophyta pollens. - Acta Bot. Acad. Sci. Hung. *18*, 307-313.

KEDVES, M., SZÓNOKY, M., MADARÁSZ, M. and KOVÁCS, G. (2000): LM and TEM investigations on the Upper Cretaceous Ajkaite, of Hungary I. - Plant Cell Biology and Development (Szeged) *12*, 9-17.

KEDVES, M., TÓTH, A. and FARKAS, E. (1991): High temperature effect on the spores of *Equisetum arvense* L. - Plant Cell Biology and Development (Szeged) *1*, 8-14.

KRUTZSCH, W. (1957): Sporen- und Pollengruppen aus der Oberkreide und dem Tertiär Mitteleuropas und ihre stratigraphische Verteilung. - Z. angew, Geol. *11/12* 509-548.

KRUTZSCH, W. (1959): Mikropaläontologische (sporenpaläontologische) Untersuchungen in der Braunkohle des Geiseltales. I. - Geologie BH. *21/22*, 1-425.

MÉDUS, J. et TRIAT, M. (1969): Le Cénomanien supérieur de la coupe de Laudun (Gard, France): Étude palynologique et données sédimentologiques. - Rev. Palaeobot., Palynol. *9*, 213-228.

PACLTOVÁ, B. and SVOBODOVÁ, M. (1992): Facial characteristics from the palynological point of view in the area of the Bohemian Cenomanian. - Paleofloristic and Paleoclimatic changes during Cretaceous and Tertiary. Geol ustav. Dionyza Stura, Bratislava, 17-24.

PANTIC, N.K. and DULIC, I.A. (1994): The study of reworked palynomorphs 1. Reworked palynomorphs in Alb-Cenomanian formation of Vojvodina and Belgrade Environs. - Ann. Geol. Penins. Balk. *58*, 101-117.

PANTIC, N.K. and DULIC, I.A. (1999): Some Contributions to the Stratigraphy of the Poganovo Paralic Series (Southeast Serbia). - Bull. T. CXIX de l'Académie serbe des sciences et des Arts, Classe des Sciences mathématiques et naturelles. Sciences naturelles N° *39*, 83-90.

POTONIÉ. R. (1956): Synopsis der Gattungen der Sporae dispersae I. Teil: Sporites. - Beih. Geol. Jb. *23*, 1-103.

POTONIÉ, R. und GELLETICH, J. (1933): Ueber Pteridophytensporen einer eozänen Braunkohle aus Dorog in Ungarn. - Sitz.-Ber. naturf. Fr. Berlin, 317-328.

SVOBODOVÁ, M. (1997): Mid-Cretaceous palynomorphs from the Blansko Graben (Czech Republic): affinities to both Tethyan and Boreal bioprovinces. - Proc. 4th EPPC Mid-Cretaceous palynomorphs from the Blansko Graben *58*, 149-156.

SVOBODOVÁ, M., MÉON, H. and PACLTOVÁ, B. (1998): Characteristics of palynospectra of the Upper Cenomanian-Lower Turonian (anoxic facies) of the Bohemian and Vocontian Basins. - Vestn-Ceského geol. ust. *73*, 229-252.

THIERGART, F. (1949): Der stratigraphische Wert mesozoischer Pollen und Sporen. - Palaeontographica *B, 89*, 1-34.

WELLMAN, C. H. (1999): Sporangia containing *Scylaspora* from the Lower Devonian of the Welsh Borderland. – Palaeontology *42*, 67–81.

WELLMAN, C.H., EDWARDS, D. and AXE, L. (1998): Ultrastructure of laevigate hilate spores in sporangia and spore masses from the Upper Silurian and Lower Devonian of the Welsh Borderland. - Phil. Trans. R. Soc. Lond. *B, 353*, 1983-2004.

WEYLAND, H. und GREIFELD, G. (1953): Über strukturbietende Blätter und pflanzliche Mikrofossilien aus den Untersenonen Tonen der Gegend von Quedlinburg. - Palaeontographica *B, 95*, 30-52.

WILSON, L.R. (1943): Elater-bearing spores from the Pennsylvanian strata of Iowa. - Amer. Midl. Nat. *30*, 518-523.

3. COMPARISON OF ULTRASTRUCTURE OF THE CUTICLE IN SOME EXTINCT AND EXTANT TAXA OF GYMNOSPERMS FROM INDIA

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Abstract

The ultrastructure of the cuticular membrane in 3 taxa of Mesozoic "pteridosperms" (cf. *Dicroidium gouldii, Komlopteris indica, Pachypteris indica*) and 4 taxa of living cycads *(Cycas circinalis, C. revoluta, Zamia fischeri, Z. furfuracea)* is reviewed and discussed. It is observed that all the 4 living species show comparable ultrastructure, while the ultrastructure in two families of Mesozoic "pteridosperms", namely, Corystospermaceae and Peltaspermaceae seems to be different.

Key words: Ultrastructure, Cuticular membrane, Gymnosperms, India.

Introduction

The plant cuticular membrane (CM) is a thin extra-cellular layer that covers most of the aerial part of the plant body, such as, the leaf, herbaceous stem, flowers, seeds and fruits. It also protects the plant against environmental stress, such as drought, high concentrations of solutes within the tissue and microbial activity. The CM is attached to the outer surface of the epidermis through an intermediate pectinaceous layer (MARTIN and JUNIPER, 1970) and transcribes features of the epidermis. The main structural components of the CM are insoluble lipid polyester cutin (HOLLOWAY, 1982), or insoluble nonhydrolyzable biomacromolecule cutan (TEGELAAR et al., 1991). Investigation of the anatomy of cuticular membrane under LM, SEM and TEM provides valuable data for identification of different taxa. The identification helps in taxonomy and further in correlation of different groups of extinct and extant taxa.

Several studies have been done on the surface morphology of CM under LM and SEM, but comparatively less data is available on the ultrastructure of CM under TEM. The CM of a large number of taxa of extant plants has been investigated at ultrastructural level (MARTIN and JUNIPER, 1970; CUTLER, ALVIN and PRICE, 1982) but not many extinct taxa of gymnosperms have been studied for the ultrastructure of CM. Thus there is a great potential for the study of ultrastructure of CM of extinct and extant taxa of gymnosperms for systematic identification, taxonomic classification and correlation of compression fossils, particularly those of the gymnosperms. A comparative study of ultrastructure of CM of extinct and extant taxa of plants may perhaps lead to identification of "the nearest living relative". This is important because "morphological and systematic similarity is assumed to reflect similarity in climatic tolerance" (MOSBRUGGER, 1999). Study of ultrastructure of fossil CM may also help in taphonomy (for example, in

determining the degree of cuticle preservation), and in understanding the evolution of the cuticles (TAYLOR, 1999).

Much more attention needs to be given to the study of ultrastructure of CM of "pteridosperms", which is a large and heterogeneous group. The cuticle of late Palaeozoic "pteridosperms", including the glossopterids, is generally poorly preserved and less resistant to chemical processing as compared to the CM of Mesozoic "pteridosperms". The latter are well preserved and exhibit structural components identifiable at ultrastructural level (ARCHANGELSKY and TAYLOR, 1986; ARCHANGELSKY, TAYLOR and KURMANN, 1986; TAYLOR, TAYLOR and ARCHANGELSKY, 1989; ARTABE and ARCHANGELSKY, 1992; BARALE and BALDONI, 1993; MAHESHWARI and BAJPAI, 1996; BAJPAI, 1997; VILLAR DE SEONE, 1997).

Information about ultrastructure of the cycadalean cuticle is known from *Encephalartos lehmannii* LEHMANN (VILLAR DE SEONE, 1997), *Stangeria paradoxa* MOORE (ARTABE and ARCHANGELSKY, 1992) and *Cycas circinalis* L. (BAJPAI, 2001).

For the present study some extinct taxa of gymnosperms were chosen to examine ultrastructural features with modern analogs by comparing similar features (for example, thickness of CM, presence or absence of lamellae, nature of CM zones, amorphous, reticulate/fibrillate, etc.). The potential diagenetic influences may also be identified. The taxa investigated belong to the Corystospermaceae (cf. *Dicroidium gouldii, Komlopteris indica, Pachypteris indica),* and the Cycadaceae *(Cycas circinalis, C. revoluta, Zamia fischeri, Z. furfuracea).*

Materials and Methods

Leaves of *Cycas circinalis, C. revoluta, Zamia fischeri* and *Z. furfuracea* were obtained from the herbarium of Birbal Sahni Institute of Palaeobotany, Lucknow. The carbonified crust of cf. *Dicroidium gouldii* was recovered from a Late Triassic grey argillaceous shale exposed in Janar rivulet, about one kilometer SSW of Harai Village in South Rewa Basin, that of *Komlopteris indica* from an Early Cretaceous grey shale in a shallow well in the village Naicolam, Cauvery Basin and of *Pachypteris indica* from an Early Cretaceous grey shale exposed near Sehora-on-Sher, Satpura Basin.

The carbonised pinnules removed from the compression fossils were treated with 40% hydrofluoric acid to digest silica. After thorough washing in triple-distilled water, the pinnules were cut into small pieces suitable for processing. Leaves of extant taxa were not given any chemical treatment at this stage. They were, however, also cut into pieces small enough for further processing. The material of both extinct and extant taxa was prosessed as per method given by MAHESHWARI and BAJPAI (1996). Final 600-700Å thin sections were cut with a diamond knife and mounted on copper grid and stained with uranyl acetate and lead citrate.

Results

cf. *Dicroidium gouldii* RETALLACK 1977 (Plate 3.1., figs. 1-3)

The CM configuration shows a three-layer configuration. The outermost polylamellate region is made up of parallel running, alternating, 4-6 electron dense, and 4-7 electron lucent lamellae, thickness of which is not uniform. Some of the lamellae are continuous while others run only for a short while. On the leaf-air interface irregular osmiophilic deposits are present, which are possibly the remnants of the epicuticular waxes left after diagenesis. Inner to polylamellate region is a comparatively thick amorphous region that shows incipient fibrillar components. The innermost zone is the thickest of the three zones and is made up of distinct fibrillae which have a "herring bone" appearance and are oriented mainly parallel to the membrane surface. At regular intervals the innermost zone forms wedge-shaped outgrowths which are taken to represent the cuticular pegs or anticlinal flanges that penetrate in inter-wall spaces between adjacent epidermal cells.

Komlopteris indica (FEISTMANTEL) BARBACKA (Plate 3.1., fig. 4)

The CM is covered with remnants of epicuticular wax at the leaf-air interface. The CM, which is not uniformly thick throughout, shows two zones, a narrow electron dense outer zone, and a comparatively much wide electron lucent homogeneous inner zone. At the innermost limit of the sub-cuticular level, where the cuticle comes in contact with the epidermis, the sub-cuticular zone forms cuticular pegs (anticlinal flanges) in which extends a narrow strip of electron dense zone. The CM conforms to Type-6 of HOLLOWAY'S (1982) cuticle types.

Pachypteris indica (OLDHAM and MORRIS) BOSE and ROY (Plate 3.1., fig. 5, Plate 3.2., fig. 1)

Randomly arranged osmiophilic bodies are seen at the leaf-air interface of the CM. The CM exhibits two distinct zones, an outer electron dense amorphous zone with homogeneous matrix, and an inner electron lucent, irregularly reticulate-fibrillate zone. The basic framework at the sub-cuticular level comprises very fine and distinctly anastomosing fibrillae. The latter branch frequently and are more numerous near the epidermal cell wall. At places several lipophilic vesicles seem to permeate the sub-cuticular level.

Cycas circinalis L. (Plate 3.2., figs. 2,3)

At the leaf-air interface a heavy deposit of epicuticular wax is seen with the thin superficial deposit of osmiophilic bodies. The CM of mature leaf exhibits a three-layered configuration. The outer well-developed polylamellate zone is made up of compactly arranged, parallel running, 8-9 electron dense lamellae alternating with 7-8 electron lucent lamellae. The lamellae are mostly uniform in width, but some lamellae run continuous while other runs only for a short distance. The polylamellate zone is followed by an amorphus homogeneous matrix that seem to form the major part of the CM. Inner to this zone is another zone that has matrix of the same density but with fine reticulations. The orientation of the reticulum is mostly parallel to the general surface of CM. Irregular masses of lipophilic secretions are seen to permeate at the sub-cuticular surface contributing to the thickness of the CM. These secretions impart an irregular dendroid appearance to the subcuticular layer.

Cycas revoluta THUNB. (Plate 3.2., fig. 4)

The CM of a mature pinna exhibits an outermost electron dense layer of epicuticular wax followed by a polylamellate zone. This zone is made up of less compactly arranged parallel running 6-7 electron dense lamellae alternating with 5-6 electron translucent

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Plate 3.1.

1-3. Cf. *Dicroidium gouldii* RETALLACK. 1. 21.000x, 2. 46.500x, 3. 168.000x.
4. *Komlopteris indica* (FEISTMANTEL) BARBACKA. 6.600x.
5. *Pachypteris indica* (OLDHAM and MORRIS) BOSE and ROY. 10.650x.

Plate 3.2.

1. *Pachypteris indica* (OLDHAM and MORRIS) BOSE and ROY. 3.600x.
2,3. *Cycas circinalis* L. 2. 10.650x, 3. 25.350x.
4. *Cycas revoluta* THUNB. 15.600x.

Plate 3.3.

1,2. *Zamia fischeri* MIQ. 1. 4.500x, 2. 10.650x.
3,4. *Zamia furfuracea* AIT. 3. 4.500x, 4. 15.600x.

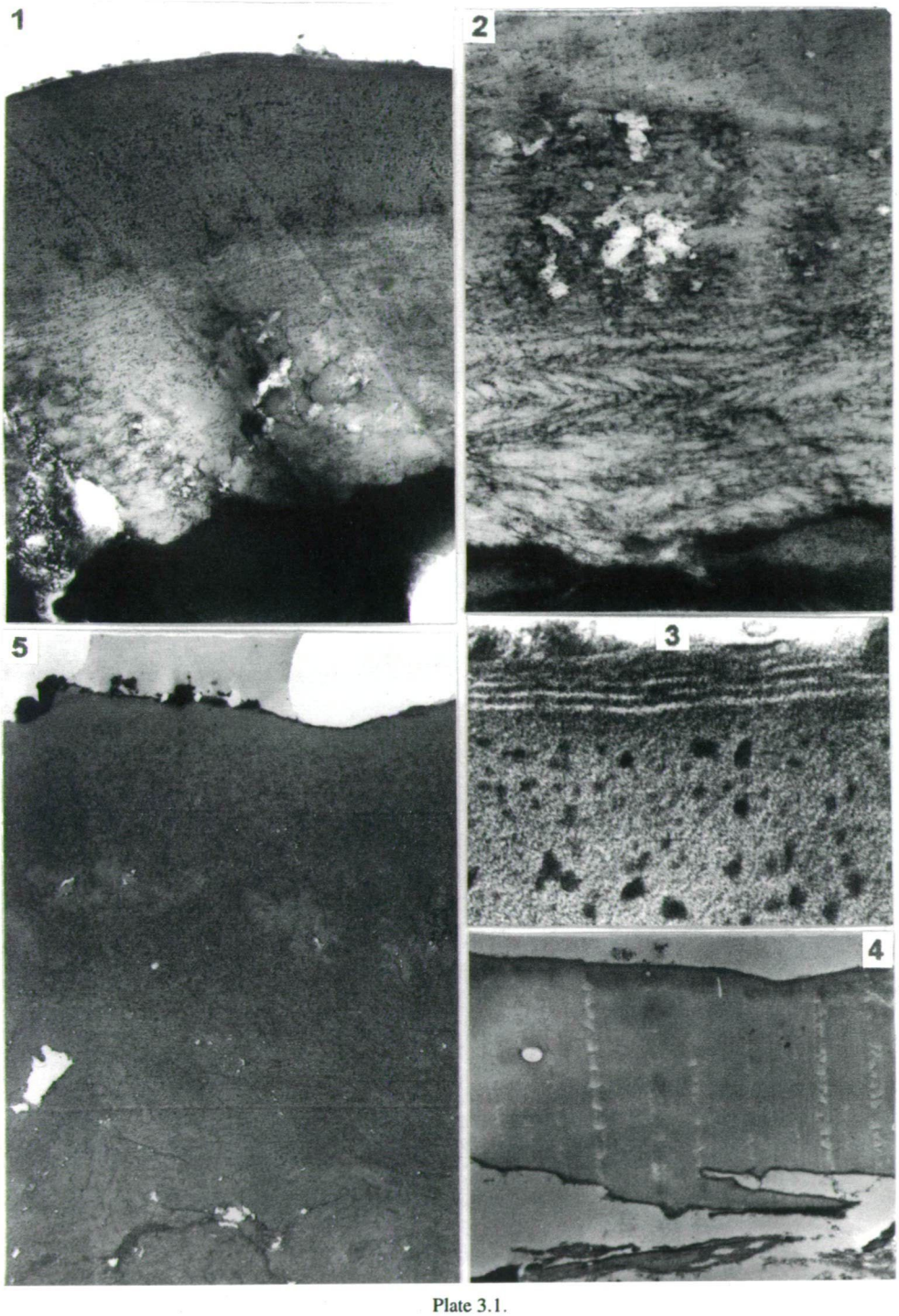

Plate 3.1.

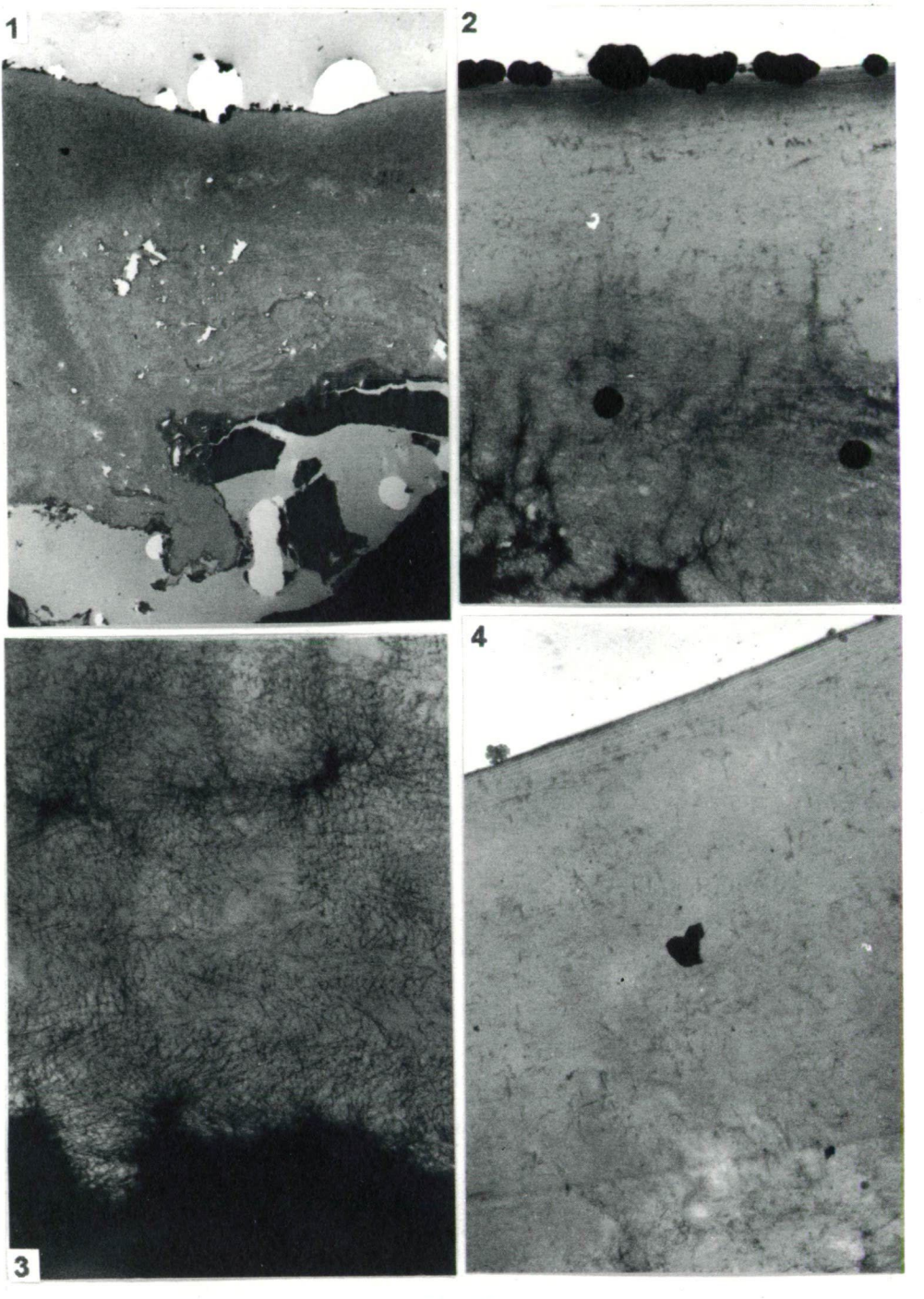

Plate 3.2.

Plate 3.3.

lamellae. A fine reticulate zone follows the polylamellate zone. At places the fine meshes in the CM are osmiophilic and have a coarse, almost channel-like appearance. At the innermost region where it adheres to the cell wall, the CM has a loose texture and appears fissured. At the base irregular masses of newly synthesized lipophilic secretions seem to permeate at sub-cuticular level.

Zamia fischeri MIQ. (Plate 3.3., figs. 1,2)

The CM is coated with epicuticular wax at the leaf-air interface. The structure of the wax layer varies from tubular, flat, fluted to at places fused along the edges and forming a thick highly electron dense layer. The outermost zone of the CM is polylamellate and is made up of robust and prominent 7-8 translucent and 8-9 opaque lamellae. The opaque lamellae vary in thickness at places. On the surface of lamellae, fine striations are present. An amorphus zone of constant homogeneous matrix follows this zone. The density of fibrillar network is very high at the junction with cell wall. The thickness of the cell wall of the epidermal cell also varies from place to place. The cytoplasm of the epidermal cell contains a well-defined nucleus with nucleolus, numerous ribosomes, endoplasmic reticulum, small vacuoles and numerous lipophilic granules.

Zamia furfuracea AIT. (Plate 3.3., figs. 3,4)

The CM is irregular in thickness, the thickness varies from one epidermal cell to other. The epicuticular wax is made up of wax flakes. At places this layer is detached from the CM. It is followed by a faintly polylamellate zone, with 4-5 lamellae. The lamellae are very loosely arranged and do not run parallel to each other. Some of the lamellae are oriented downwards towards the periclinal walls of the epidermal cells. The rest of the zone is structurally homogeneous. The basal portion of the CM attached to the surface of epidermal cell is electron dense zone because of the compactly arranged fibrillae.

Discussion and Conclusions

The cuticular membrane in all the four species of extant cycads investigated shows a polylamellate outermost zone, though faintly so in *Zamia furfuracea*. On the other hand, of the three species of extinct "pteridosperms", only that of the Corystospermaceae shows a polylamellate outermost zone. This zone is absent in the two species of the Peltaspermaceae. Apparently the taxa of Corystospermaceae and Peltaspermaceae (both Mesozoic "pteridosperms") may not be closely related. However, more taxa need to be investigated before arriving at a definite conclusion. The "herring bone" structure seen in the CM of cf. *Dicroidium gouldii* is assumed to have developed under stress conditions as the plant was living in an arid environment.

The fibrillate nature of the inner zone in the CM of *Pachypteris indica* as compared to electron lucent homogeneous inner zone of *Komlopteris (Thinnfeldia) indica* indicates that the two taxa are different and may not belong to the same genus as believed by certain authors. The lipophilic bodies or vesicles observed in *Pachypteris indica* possibly added to the structural thickness of the CM as has been observed in extant *Cycas revoluta*.

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References

ARCHANGELSKY, A., ANDREIS, R.R., ARCHANGELSKY, S. and ARTABE, A. (1995): Cuticular characters adapted to volcanic stress in a new Cretaceous cycad leaf from Patagonia, Argentina. Considerations on the stratigraphy and depositional history of the Bacquero Formation. - Rev. Palaeobot. Palynol. *89*, 213-233.

ARCHANGELSKY, S. and TAYLOR, T.N. (1986): Ultrastructural studies of fossil plant cuticles. II. *Tarphyderma* gen. n., a Cretaceous conifer from Argentina. - Am. J. Bot. *73*, 1577-1587.

ARCHANGELSKY, S., TAYLOR, T.N. and KURMANN, M.H. (1986): Ultrastructural studies of fossil plant cuticles: *Ticoa harrisii* from the Early Cretaceous of Argentina. - Bot. J. Linn. Soc. *92*, 101-116.

ARTABE, A.E. and ARCHANGELSKY, S. (1992): Las Cycadales *Mesodescolea* ARCHANGELSKY emend. ARCHANGELSKY y PETRIELLA 1971 (Cretácico) y *Stangeria* MOORE (actuel). - Ameghiniana *29*, 115-123.

BAJPAI, USHA (1997): Taphonomic constraints on preservation of cuticle in compression fossils: fungi induced ultrastructural changes in cuticular membrane. - Palaeobonanist *46*, 31-34.

BAJPAI, USHA (2001): Ultrastructure of the leaf cuticle in *Cycas circinalis* LINN. - The Paleobotanist (in press).

BARALE, G. and BALDONI, A. (1993): L'ultrastructure de la cuticle de quelques Bennettitales du Crétacé inférieur d'Argentine. - C. R. Acad. Sci. Paris, Sér. II *316*, 1171-1177.

CUTLER, D.F., ALVIN, K.L. and PRICE, C.E. (1982): The Plant Cuticle. - Academic Press, London.

HOLLOWAY, P.J. (1982): Structure and histochemistry of plant cuticular membranes: an overview. In: The Plant Cuticle, eds.: CUTLER, D.F., ALVIN, K.L. and PRICE, C.E. - Academic Press, London, 1-32.

MAHESHWARI, H.K. and BAJPAI, USHA (1996): Biochemical degradation of the cuticular membrane in an Early Cretaceous frond: a TEM study. Current Science *70*, 933-935.

MAHESHWARI, H.K. and BAJPAI, USHA (1996): Ultrastructure of the "cuticular membrane" in two Late Triassic corystospermaceous taxa from India. - Palaeobotanist *45*, 41-49.

MARTIN, J.T. and JUNIPER, B.E. (1970): The Cuticle of Plants, - Edward Arnold, London.

MOSBRUGGER, V. (1999): The nearest living relative method. In: Fossil plants and spores: modern techniques, eds.: JONES, T.P. and ROWE, N.P. - Geological Society. London, 261-265.

TAYLOR, W.A., TAYLOR, T.N. and ARCHANGELSKY, S. (1989): Comparative ultrastructure of fossil and living gymnosperm cuticles. - Rev. Palaeobot. Palynol. *59*, 145-151.

TAYLOR, T.N. (1999): The ultrastructure of fossil cuticle. In: Fossil plants and spores: modern techniques, eds.: JONES, T.P. and ROWE, N.P. - Geological Society, London, 113-115.

TEGELAAR, E.W., KERP, J.H.F., VISSHER, H., SCHENCK, P.A. and DE LEEUW, J.W. (1991): Bias of the paleobotanical record as a consequence of variations in chemical composition of higher vascular plant cuticles. – Paleobiology *17*, 133-144.

VILLAR DE SEONE, L. (1997): Estudio cuticular comparado de nuveas Cycadales de la Formatiòn Baquerò (Cretàcico inferior), Provincia de Santa Cruz, Argentina. - Revista Española Paleont. *12*, 129-140.

4. TRANSMISSION ELECTRON MICROSCOPY OF PARTIALLY DEGRADED TELIOSPORES OF USTILAGO MAYDIS (DE CANDOLLE) CORDA

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Abstract

During our experimental studies on the ultrastructure of the teliospores of *Ustilago maydis* 2-aminoethanol, $KMnO_4$ 1% and acetic anhydride were used as organic solvents and oxidizing agents. Important alterations were observed at the fine structure of the wall, and in the inner part of the teliospore. Similarities were established between the ultrastructural data of experiment No.: 191 (2-aminoethanol, $KMnO_4$ and acetic anhydride) and the X-ray irradiated teliospores over 15 minutes.

Key words: Palynology, recent, *Ustilago maydis*, partial degradation, TEM.

Introduction

In the course of our LM and TEM research programs on the partial dissoluted, degraded and irradiated sporomorphs, we have established important differences in the wall biopolymer resistance of the different taxa of the spores and pollen grains, cf. KEDVES and GÁSPÁR (1994), KEDVES and UNGVÁRI (1996), KEDVES, KÁROSSY and BORBOLA (1997), KEDVES et al. (1998). Biopolymer and molecular structures were discovered in the walls of the sporomorphs after partial degradation with different experimental influences. The protective function of the melanins in the spore wall was pointed out and/or discussed in several papers, e.g.. PIROZYNSKI (1977). At the teliospores of *Ustilago maydis* the X-ray irradiation demonstrated the protective function of the melanins in the wall, moreover molecular structures sensu strictu were also observed by KEDVES, PÁRDUTZ and BORBOLA (1998).

The aim of this paper is to present the TEM results of the partially degraded teliospores of *Ustilago maydis* in comparison with the results of the irradiated spores.

Materials and Methods

The material of investigation was collected by Dr. Á. PALÁGYI on 22.08.1981. The spores were frozen at -20°C after collection. The temperature of partial degradation experiments was 30°C, the different methods were as follows:

Experiment No.: 188. - 20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hours.

Experiment No.: 189. - 20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hours, after washing + 10 ml $KMnO_4$ 1%, during 24 hours.

Experiment No.: 190. - 20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hours, after washing + 10 ml $KMnO_4$ 1% during 48 hours.

Experiment No:. 191. - 20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hours, after washing + 10 ml $KMnO_4$ 1% during 24 hours, washing again + 2 ml acetic anhydride, length of time: 24 hours.

After experiment the spores were postfixed with OsO_4 aq. dil. 1% and embedded in Araldite. The ultrathin sections were made with glass knives on a Porter Blum ultramicrotome in the Electron Microscopical Laboratory of the Institute of Biophysics of the Biological Center of Hungarian Academy of Sciences. TEM pictures were taken in a Tesla BS-540, resolution 6-7 Å. All pictures are unretouched.

Results

Experiment No.: 188. (Plate 4.1., figs. 1,2). The exospore of the wall was partially degraded. Electron dense globular units were observed within the substance of the wall. Important degradations were observed in the fine structure of the exospore (Plate 4.1., fig. 2). The endospore swolled.

Experiment No.: 189. (Plate 4.1., figs. 3,4). The fine structure of the exospore was highly degraded, sometimes completely disappeared. The epispore remain in a good condition, the endospore swolled. The protoplasm was extremely contracted (Plate 4.1., fig. 3).

Experiment No.: 190. (Plate 4.1., figs. 5,6). Under the hardly degraded exospore a thin epispore layer was observed. The endospore is degraded. Electron dense degraded protoplasm is in the middle of the teliospore (Plate 4.1., fig. 5).

Experiment No.: 191. (Plate 4.2., figs. 1-3). The results of this experiment are extremely interesting. The exospore is superficially degraded. This degradation process revealed globular biopolymer units on the outer and inner surface of this layer by; the degradation of the further layers are not uniform. Under the exospore the finely granular epispore was more or less well preserved (Plate 4.2., fig. 1). Sometimes this part of the wall seemed to be two layered. Electron dense endospore may be present. Sometimes the inner part of the endospore is characteristically electron dense (Plate 4.2., fig. 2). As such after the experiment, this part of the wall appears to be three layered.

Discussion and Conclusions

1. Among the LM results it is necessary emphasize that, based on our previous investigations (KEDVES and GÁSPÁR, 1994), the teliospores of *Ustilago maydis* were extremely resistant to dissolution with diethylamine and merkapto-ethanol. The thick exospore of *Equisetum arvense* L. and the sporopollenin of the pollen grains of *Quercus robur* L. dissolute with the above mentioned agents.

Plate 4.1.

1-6. *Ustilago maydis* (DE CANDOLLE) CORDA. TEM pictures of partially degraded teliospores.
1,2. Experiment No.: 188, 1. Negative No.: 6955, 10.000x, 2. Negative No.: 6340, 50.000x.
3,4. Experiment No.: 189, 3. Negative No.: 6960, 10.000x, 4. Negative No.: 6961, 50.000x.
5,6. Experiment No.: 190, 5. Negative No.: 6376, 10.000x, 6. Negative No.: 6379, 50.000x.
Exs = exospore, Eps = epispore, Ens = endospore, Cp = cytoplasm.

Plate 4.2.

1-3. *Ustilago maydis* (DE CANDOLLE) CORDA. TEM pictures of partially degraded teliospores. Experiment No.: 191, 1. Negative No.: 6618, 25.000x, 2. Negative No.: 6616, 50.000x, 3. Negative No.: 6617, 50.000x.
Exs = exospore, Eps = epispore, Ens = endospore.

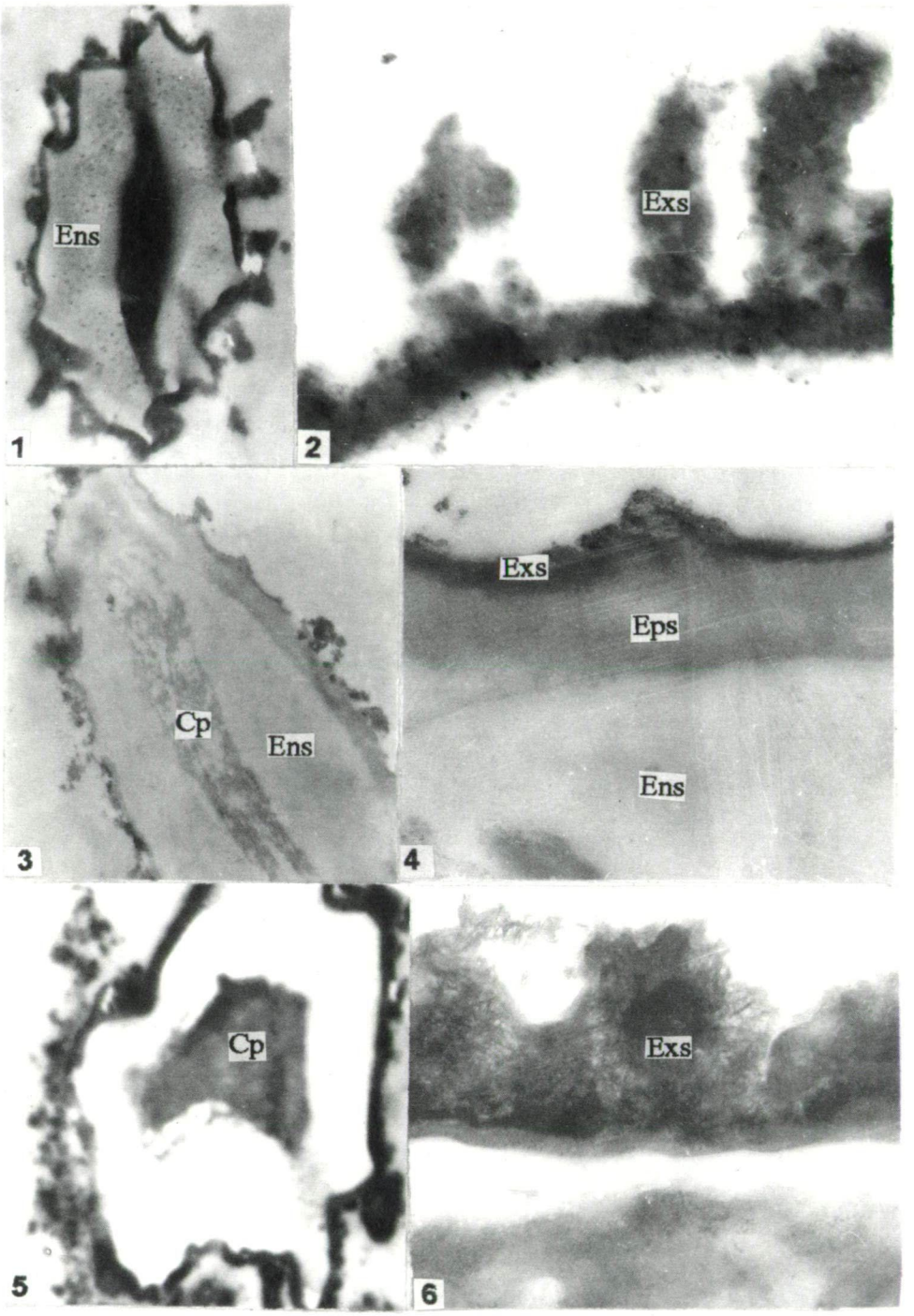

Plate 4.1.

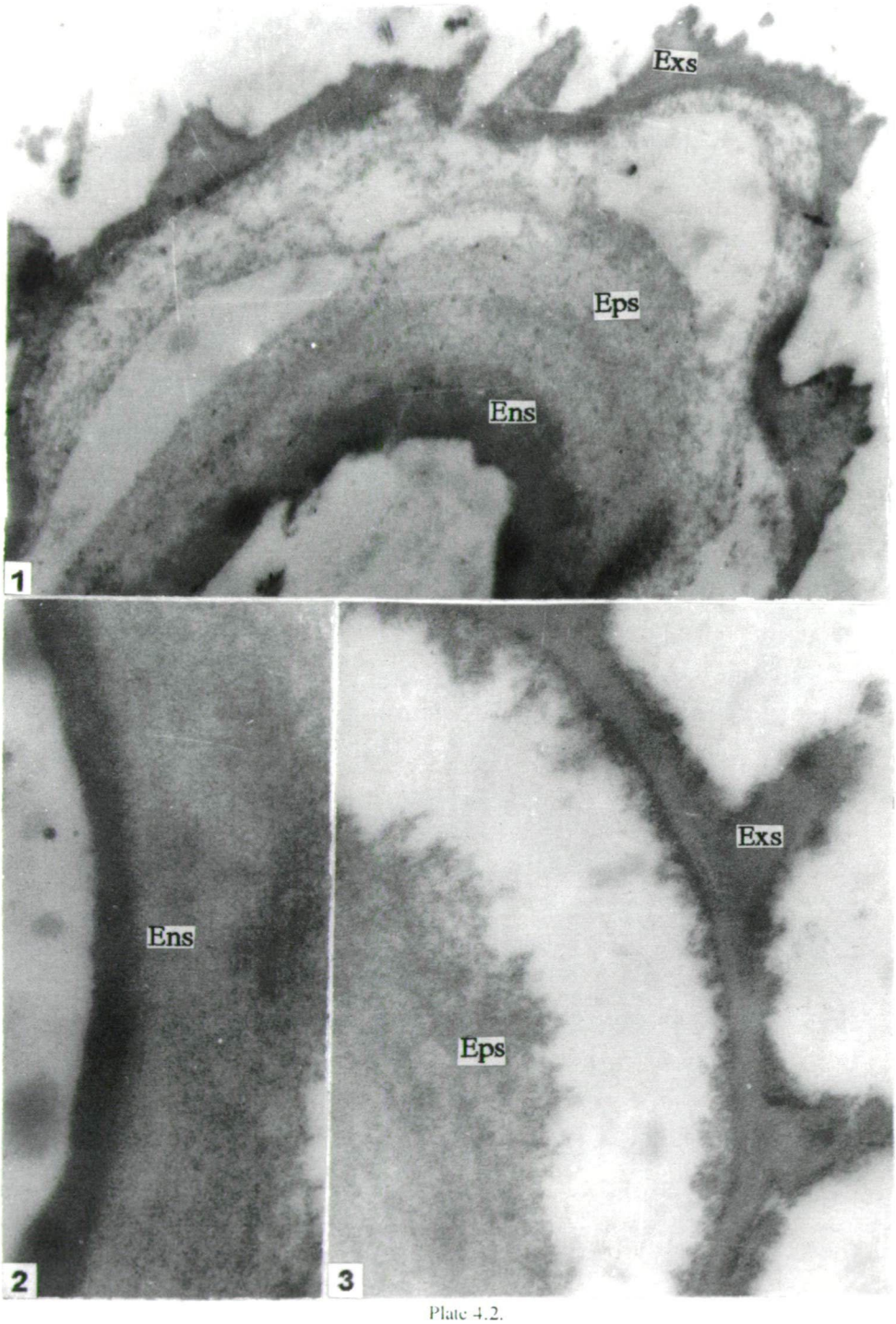

Plate 4.2.

2. By TEM method, the partial degradation experiments revealed different kinds of biopolymer structures in the wall layers of the teliospores of *Ustilago maydis*. Globular biopolymer units were observed on the surfaces. Presumably the TEM method is also a factor of the partial degradation.

3. Sometimes the TEM data within one experiment were not completely identical. This may be consequence of the state of maturity of the spores not being completely on the same level or was not in a fertile state.

4. It is interesting that the TEM results of the experiment No.: 191 are similar to or more or less identical with the X-ray irradiated teliospores over 15 minutes.

5. It may be pointed out, that the application of the acetic anhydride as degradation agents may be useful again for the further partial degradation experiments.

Acknowledgements

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References

KEDVES, M. and GÁSPÁR, I. (1994): Les alterations sécondaires des spores et des grains de pollen dissous partiellement. - Trabajos de Palinología básica y aplicada, X Simposio de Palinología (A.P.L.E.), Univ. de Valencia 1994, 153-161.

KEDVES, M., HORVÁTH, E., MÉSZÁROS, E., MÉSZÁROS, R., RONTÓ, G., SZLÁVIK, N., GAUDÉNYI, SZ. and KALMÁR, Á. (1998): LM investigations of partially dissolved sporomorphs II. - Plant Cell Biology and Development (Szeged) *9*, 76-87.

KEDVES, M., KÁROSSY, Á. and BORBOLA, A. (1997): LM investigations of partially dissolved sporomorphs I. - Plant Cell Biology and Development (Szeged) *8*, 44-55.

KEDVES, M., PÁRDUTZ, Á. and BORBOLA, A. (1998): Transmission electron microscopy of X-ray irradiated teliospores of *Ustilago maydis*. - Grana *37*, 29-34.

KEDVES, M. and UNGVÁRI, E. (1996): X-ray effect to the LM morphology of some gymnosperm and angiosperm pollen grains. - Plant Cell Biology and Development (Szeged) *7*, 75-81.

PIROZYNSKI, K.A. (1977): Melanins as palaeobiological and paleoenvironmental indicators? - Syllogeus *12*, 59-62.

5. LM AND SEM INVESTIGATIONS ON PARTIALLY DISSOLVED ALLERGEN POLLEN GRAINS II.

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Abstract

Pollen grains of *Taxus baccata* L. were investigated with LM and SEM methods after dissolution with 2-aminoethanol for 30 minutes, 1 hour, 5, 10 and 24 hours. Alterations in the LM morphological characteristic features and the degradation processes of the submicroscopic superficial ornamentation and the ectexine layers are presented. Special attention was made to the alterations, which happened as a consequence of the swelling of the intine.

Key words: Experimental, Palynology, recent, *Taxus baccata*, LM, SEM

Introduction

Pollen grains of *Taxus baccata* have been recognized as allergenic pollen grains, e.g.; PEHLIVAN (1995), LEJOLY-GABRIEL (1986), JÁRAI-KOMLÓDI and MEDZIHRADSZKY (1993). There are several publications concerning this pollen grains in different ways. Alterations as a consequence of hydration were recognized by several authors. SOUTHWORTH (1986) pointed out that in certain Coniferophytina (Pinaceae, Cupressaceae, Taxaceae and Taxodiaceae) the exine separates from the intine and protoplasm. In this regard, she cited several papers: WODEHOUSE, (1935), MÜLLER-STOLL, (1948), VASIL and SAHNI, (1964), DUHOUX (1972, 1982), VILLAR, KNOX and DUMAS, (1984). In this paper she emphasized that purified exines can be isolated for chemical analysis by hydration. During our experimental investigations on different kinds of inaperturate pollen grains, we observed the same phenomenon as a consequence of the effect of X-rays (KEDVES and UNGVÁRI, 1996) and after partial dissolution by different organic solvents (KEDVES, KÁROSSY and BORBOLA, 1997). In this paper the term of the Duhoux effect was introduced. Later this effect was investigated on inaperturate gymnosperm and angiosperm pollen grains by KEDVES et al. (2000). In this paper the ultrastructure of X-ray irradiated and hydrated pollen grains of *Taxus baccata* were also published. In this regard, the papers of DUHOUX (1972, 1975, 1979) were used. The high temperature effect on some inaperturate gymnosperm pollen grains (*Juniperus virginiana* L., *Taxus baccata* L.) was also used. The most important alterations of the pollen grains of *Taxus baccata* are the plicate "Normapolles-like" types. At the terminal parts of the "plicae" pores or pseudopores may also occur. Two secondary types may be distinguished, the so-called "*Plicapollis* form" and the "*Interpollis* type".

Another problem, that is the distinction of the Cupressaceae and Taxaceae pollen grains, was pointed out by BELMONTE et al. (1999) as follows; p. 39: "The pollen grains from Cupressaceae cannot be differentiated under light microscopes, even at the genus level, nor can the pollen grains of Taxaceae (*Taxus baccata*, native to the Iberian peninsula) or Taxodiaceae (*Cryptomeria japonica*, frequently planted as an ornamental). In the aerobiological studies, all these taxa appear under the name Cupressaceae."

The results of UENO (1974) concerning the inaperturate gymnosperm pollen grains are important. Ectexine lost pollen grains of *Cryptomeria japonica* germinated under in vitro conditions in consequence of different carbohydrates.

There are several EM data. TAKEOKA (1966) investigated the surface of the pollen grains of *Taxus cuspidata* by using the methylmethacrilate carbon two-staged replica method. A SEM picture from *Taxus cuspidata* SIEB. et ZUCC. var *nana* REHD. was published by MIYOSHI (1980). Further SEM data were published by XI YI-ZHEN (1986) from *Taxus chinensis* (PILGER) REHD., *T. cuspidata* SIEB. et ZUCC. and *T. yunnanensis* CHENG et L.K. FU. TEM data from *Taxus yunnanensis* were published in the paper of XI YI-ZHEN (1986).

Experimental TEM data from the pollen grains of *Taxus baccata* were published by KEDVES (1987a,b). Partially degraded and fragmented exines, using a magnetic stirrer, of *Juniperus virginiana* L. and *Taxus baccata* L. were investigated with the transmission electron microscope. Several kinds of levels of the biopolymer structure of the sporopollenin were observed, including the basic regular pentagon and also the so-called Penrose units.

Materials and Methods

The material for investigation was collected by Miss M. MADARÁSZ on the 07.03.2000. Locality: Szeged, Honvéd Square cultivated. Non-experimental fresh (T-12-21) and partially degraded pollen grains were investigated, 1 ml 2-aminoethanol was added to 5 mg pollen grains. Temperature: 30 °C, length of time: 30 minutes (T-12-22), 1 hour (T-12-23), 5 hours (T-12-24), 10 hours (T-12-25) and 24 hours (T-12-26). Non coloured pollen grains (a) and stained with Methylviolet (b) were mounted in glycerine-jelly, hydrated to 39.6%. The morphological alterations, the diameter of the pollen grains and the ratio of the diameter/intine thickness were investigated with the LM method. For scanning electron microscopical investigations the dry pollen grains were covered with gold-palladium. The pictures were taken in the SEM Laboratory of the Department of Botany of the University of Szeged on a Hitachi S-2400 instrument, resolution about 40 Å. All pictures are unretouched.

Results

LM results (Plate 5.1., figs. 1,2,5,6,9,10, plate 5.2., figs. 1,2,5,6,10,11)

The climate was extremely unusual during the autumn of 1999, and the winter of 2000. Relatively mild periods started the growth of staminate flowers and cooler days stopped this development. This kind of change in the temperature repeated several times during the final development of the flowers to the stage of mature pollen grains. Without doubt, this was the reason that in the slides several aberrant forms of pollen grains were observed. This question may be the subject of another investigation, in this place the relative abundance of the "normally" aberrant dyads may be emphasized. One pollen grain was relative big, the other one was small and probably not fertile.

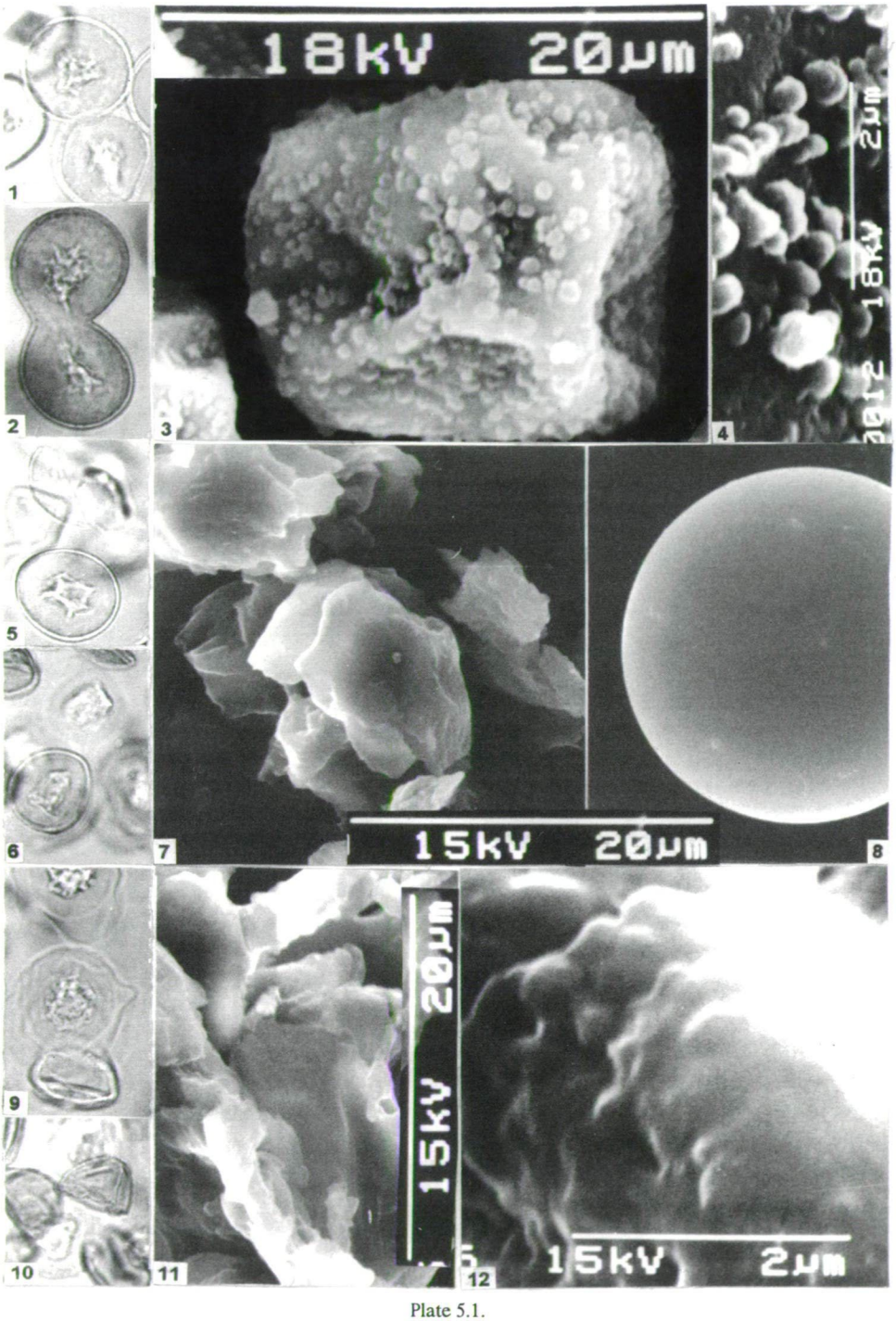
1
2
18kV 20µm
3
4
2µm 18kV
5
6
7
15kV 20µm
8
9
10
11
15kV 20µm
12
15kV 2µm

Plate 5.1.

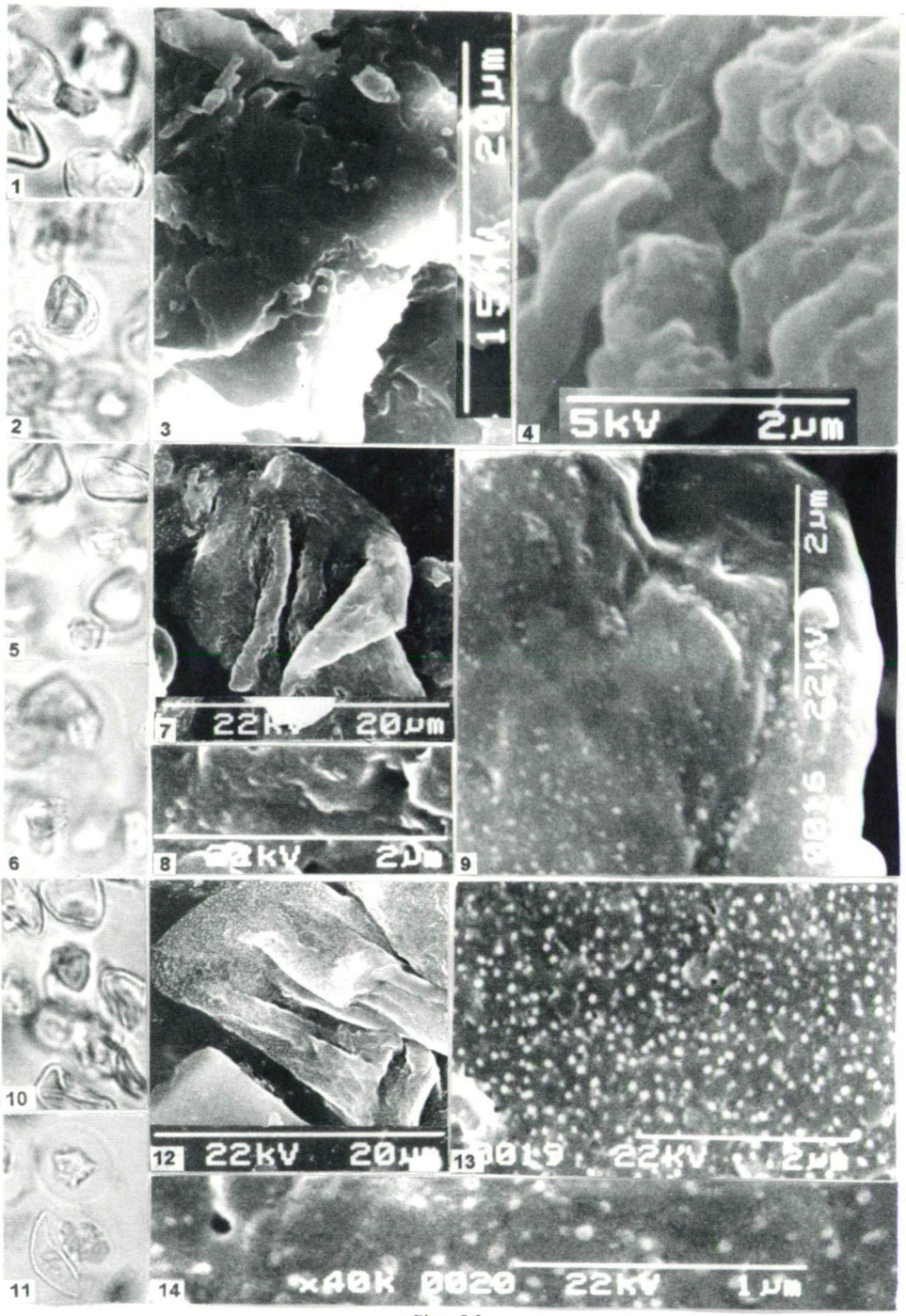
1
2
3
4 5kV 2µm
5
6
7 22kV 20µm
8
9 2µm
10
11
12 22kV 20µm
13 22kV 2µm
14 ×40K 0020 22kV 1µm

Plate 5.2.

Plate 5.1.

Figs. 1- 12. *Taxus baccata* L.
Figs. 1-4. Fresh (non-experimental) pollen grains.
Figs. 1,2. LM pictures, fig. 1. unstained (T-12-21, a), fig. 2. stained pollen grain with Methylviolet (T-12-21, b), 750.x.
Figs. 3,4. SEM pictures, fig. 3, illustrate a general survey of the surface of the pollen grains with orbiculi, fig. 4.a highly magnified picture of the orbiculi and the finely granular surface of the ectexine.
Figs. 5-8. Partially degraded pollen grains with 2-aminoethanol during 30 minutes (T- 12-22).
Figs. 5,6. LM pictures, fig. 5. unstained (a). fig. 6. stained pollen grains with Methylviolet.(b), 750x.
Figs. 7,8. SEM pictures, fig. 7. illustrate the disappearance of the orbiculi, and the fragility of the exine. The greatest part of the pollen grains was broken under the SEM method. Fig. 8 was taken from the inner body (ectexine lost) pollen grain.
Figs. 9-12. Partially degraded pollen grains during 1 hour (T-12-23).
Figs. 9,10. LM pictures, fig. 9. unstained, fig. 10. stained pollen grains with Methylviolet, 750x.
Figs. 11,12. SEM pictures, fig. 11. illustrate the damaged and broken ectexine. Fig. 12, in this highly magnified picture the degradation of the outest part of the ectexine is represented.

Plate 5.2.

Figs. 1-14. *Taxus baccata* L.
Figs. 1-4. Partially degraded pollen grains with 2-aminoethanol during 5 hours.(T-12-24).
Figs. 1,2. LM pictures, fig. 1 unstained (a), fig. 2. stained pollen grains with Methylviolet (b), 750x.
Figs. 3,4. SEM pictures, fig. 3 illustrate the degraded and brokened ectexine, in the highly magnified picture (fig. 4) the details of the degraded outer surface of the ectexine are well shown.
Figs. 5-9. Partially degraded pollen grains with 2-aminoethanol during 10 hours (T-12-25).
Figs. 5,6. LM pictures, fig. 5 unstained (a), fig. 6. stained pollen grains with Methylviolet (b), 750x.
Figs. 7-9. SEM pictures, fig. 7 illustrate a "hiatus form" In the highly magnified picture (fig. 9) small granular ornamental elements appeared.
Figs. 10-14. Partially degraded pollen grains with 2-aminoethanol during 24 hours (T-12-26).
Figs. 10,11. LM pictures, fig. 10 unstained (a), fig. 11. stained pollen grains with Methylviolet (b), 750x.
Figs.12-14. SEM pictures, fig. 12 illustrate a "hiatus form", with tiny granules on the surface. In highly magnified pictures (figs. 13, 14) the small granules are better illustrated.

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1. Morphological alterations of the pollen grains during the experiments

Explanation: a - fresh, b - coloured pollen grains,1. - pollen grains with ectexine, 2. - opened ectexine with intine and protoplasm, 3. - ectexine lost pollen grains, 4. - the empty, ectexine, hiatus form, 5. - different kinds of aberrant forms.

1		2		3		4		5		Experiment No
a	b	a	b	a	b	a	b	a	b	
90.0	37.0	0.0	0.0	0.0	35.0	0.0	21.0	10.0	7.0	T-12-21
6.5	6.0	0.0	1.0	43.0	60.0	50.5	33.0	0.0	0.0	T-12-22
4.0	3.5	0.0	0.0	47.5	50.0	48.5	46.0	0.0	0.5	T-12-23
3.5	1.5	0.0	0.5	54.0	51.0	42.5	47.0	0.0	0.0	T-12-24
3.0	1.5	0.0	0.0	47.5	56.5	49.5	42.0	0.0	0.0	T-12-25
3.5	1.5	0.0	0.0	39.0	63.0	57.5	35.0	0.0	0.5	T-12-26

It is well shown that the stain changed the morphology of the pollen grains. In contrast to the 90% of the unstained pollen grains with ectexine, this form is represented with only 37.0% at the stained ones. The effect of the 2-aminoethanol resulted in large numbers of the ectexine lost pollen grains (inner bodies) and the so-called hiatus forms. The quantity of the two forms must be theoretically equal. Important differences were observed in experiment No.: T-12-26 with the coloured pollen grains. It is worth mentioning that the aberrant forms seem to be less resistant against staining and dissolution with 2-aminoethanol. 10% was observed in the slides of the fresh, unstained pollen grains, 7.0% in the stained and the maximum in the dissoluted material was 0.5%.

2. Diameter of the pollen grains

Fresh (non-coloured) pollen grains (a)

17.5	20.0	22.5	25.0	27.5	30.0	32.5	35.0	37.5	µm	Experiment No
		2.0	15.5	33.0	31.0	14.5	3.0	1.0	%	T-12-21
	12.0	26.5	32.0	22.0	6.5	1.0				T-12-22
2.5	17.0	32.0	32.0	13.0	3.5					T-12-23
0.5	26.5	30.5	30.0	9.5	2.5	0.5				T-12-24
1.5	18.5	35.5	27.5	13.0	2.0	1.5				T-12-25
0.5	12.5	31.5	27.5	16.0	11.0	1.0				T-12-26

The diameter of the pollen grains diminishes during the partial degradation.

Stained pollen grains (b)

17.5	20.0	22.5	25.0	27.5	30.0	32.5	35.0 µm		
	3.0	20.0	42.5	27.5	4.5	2.5		%	T-12-21
1.0	14.5	27.5	30.5	17.0	9.0	0.5			T-12-22
	8.5	22.0	34.5	24.5	8.5	1.5	0.5		T-12-23
2.0	21.5	31.5	24.0	15.0	4.5	1.5			T-12-24
2.5	17.5	23.5	32.0	15.5	7.0	1.0			T-12-25
	21.0	33.0	25.0	12.0	7.0	2.0			T-12-26

In the stained pollen grains, nearly the same trend was observed as with the unstained ones.

3. Ratios of diameter/intine thickness

With the non-stained and stained pollen grains, this ratio increased more or less regularly. It is worth mentioning that with the non-experimental pollen grains there are differences in the percentages of the stained and unstained pollen grains.

SEM results (Plate 5.1., figs. 3,4,7,8,11,12, plate 5.2., figs. 3,4,7,9,12-14)

The characteristic orbicules were observed exclusively on the non-experimental material (Plate 5.1., figs. 3,4). After 30 minutes of dissolution important alterations happened in the basic morphology (Plate 5.1., fig. 7). An ectexine lost so-called "inner body" of the pollen grain was also observed. The surface seems to be finely punctate/granulate. Results after experiments during 1 and 5 hours (Plate 5.1., figs. 11,12) and (Plate 5.2., figs. 3,4) respectively are essentially identical. Secondarily rugulate-verrucate sculpture appeared on the surface of the strongly damaged ectexine. (Plate 5.1., fig.12, plate 5.2., fig. 4). Relatively numerous "hiatus forms" were observed after dissolution during 10 and 24 hours (Plate 5.2., figs. 7, 12). In the highly magnified pictures small globular units appeared, which may be larger biopolymer units or the elements of the middle part of the ectexine.

%	2	2.16	2.2	2.25	2.4	2.5	2.6	2.75	2.8	3.0	3.25	3.3	3.5	3.6	4.0	4.3	4.5	5.0	5.5	6.0	7.0	8.0
T–12–21 a		0.5	1.0		2.5	4.5	3.0	19.0	2.5	21.5	10.0	11.5	0.5	13.0	7.5	1.5	1.0	0.5				
T–12–22 a			0.5			4.5	1.5	3.0		13.0	0.5	20.0		16.0	14.0	0.5	15.0	7.5	3.5	0.5		
T–12–23 a						2.0	3.0	2.5		16.0		22.5	2.5	9.0	15.5		18.0	8.0	1.0			
T–12–24 a	0.5			3.5	1.0	5.5	5.5	7.0		10.0	0.5	16.5	0.5	2.0	20.5		18.0	7.5	1.0			0.5
T–12–25 a			0.5			0.5	1.0	3.5		8.5	1.0	11.5	0.5	7.5	17.5	0.5	28.5	15.5	2.0		1.0	0.5
T–12–26 a			0.5			0.5	0.5	4.5		12.5	0.5	11.5	0.5	8.0	16.0		24.5	15.5	3.0	0.5		1.5
T–12–21 b	1.5		1.0	4.5		21.0	2.5	18.5		16.0	1.0	18.0		8.0	4.0		1.5	2.5				
T–12–22 b			0.5		0.5	0.5	2.0	5.0		17.0	0.5	18.0	1.0	5.0	18.0		15.0	14.0	2.0	1.0		
T–12–23 b						0.5		2.5		17.5	1.0	22.0	0.5	19.0	13.5		17.5	12.5	3.0	0.5		
T–12–24 b					1.0		1.0	5.0		10.5	1.0	10.0	2.0	7.0	22.5		22.5	14.5	3.0			
T–12–25 b	0.5				2.0	3.5	3.0	6.0		10.0		12.5	1.0	8.5	15.0		16.5	15.5	1.0	0.5	2.0	2.5
T–12–26 b								1.0		10.0	2.0	12.0		10.0	22.0		25.0	13.0	2.0	2.0		1.0

Discussion and Conclusions

1. Based on our present results we observed the effect of climatic factors on the ontogenesis of the pollen grains of *Taxus baccata*. In consequence of the unusual changes in temperature during the autumn of 1999 and the winter of 2000, several kinds of aberrant forms were observed in the fresh pollen material. It is worth mentioning that after dissolution with 2-aminoethanol, the aberrant forms disappeared, so we can presume, that the molecular structure of the sporopollenin of these forms is less resistant. This may be a disturbed ontogenetical stage, which disturbed the biosynthesis or the precursors or the polymerization processes.

2. The stain used and partial degradation altered the LM morphological characters of the pollen grains of *Taxus baccata*.

3.From the SEM data, we can point in the first place to the disappearance of the orbiculi after 30 minutes of degradation. In the degradation process of the ectexine, two major stages may be distinguished.

3.1. Pollen grains treated during 1-5 hours may be characterized by the uneven surface of the ectexine without orbiculi.

3.2. After 10 and 24 hours of treatment, the tiny granules on the more or less smooth surfaces of the pollen grains are characteristic. These tiny granules may be larger globular molecular units or elements of the inner part of the ectexine.

Finally we can conclude that the ecological conditions may be taken in consideration, to this we can cite one of our papers concerning the solution of the sporopollenin of the ectexine of the pollen grains of *Quercus*.(KEDVES and GÁSPÁR, 1996).

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References

BELMONTE, J., CANELA, M., GUARDIA, R., GUARDIA, R.A., SBAI, L., VENDRELL, M., CARIÑANOS, P., DIAZ DE LA GUARDIA, C., DOPAZO, A., FERNÁNDEZ, D., GUTTIÉRREZ, M. and TRIGO, M.M. (1999): Aerobiological Dynamics of the Cupressaceae pollen in Spain, 1992-98. - Polen *10*, 27-38.

DUHOUX, E. (1972): Evolution structurale de la paroi du grain de pollen de *Juniperus communis* L. (Cupressales), cultivé in vitro, au cours de la phase d'hydratation. - C.R. Acd. Sc. Paris, *274*, 2767-2770.

DUHOUX, E. (1975): L'aperture dans l'exine et dans l'intine externe du pollen de *Juniperus chinensis* L., et de *Juniperus communis* L. - Pollen et Spores *17*, 191-201.

DUHOUX, E. (1979): Mécanisme de la rupture de l'exine du pollen hydraté de quelques Gymnospermes. - 104e Congres national des sociétés savantes, 1979, Bordeaux 1979, sciences II, 117-127.

DUHOUX, E. (1982): Mechanism of exine rupture in hydrated taxodioid type of pollen. - Grana *21*, 1-7.

JÁRAI-KOMLÓDI, M. and MEDZIHRADSZKY, ZS. (1993): Aeropollen research in the air of Budapest in 1992. - Studia Botanica Hungaricaa *24*, 45-49.

KEDVES, M. (1987a): In vitro destruction of the exine of recent palynomorphs II. - Acta Biol. Szeged. *33*, 49-56.

KEDVES, M. (1987b): Higher organized sporopollenin biopolymer structures and the explosion under scanning effect. - Acra Biol. Szeged. *33*, 163-165.

KEDVES, M. and GÁSPÁR, I. (1996): New data concerning the solubility of the pollen grains of the genus *Quercus* L. - Plant Cell Biology and Development (Szeged) *7*, 56-61.

KEDVES, M., KÁROSSY, Á. and BORBOLA, A. (1997): LM investigations of partially dissolved sporomorphs I. - Plant Cell Biology and Development (Szeged), *8*, 44-55.

KEDVES, M., MADARÁSZ, M., SZÉCSÉNYI, A., HORVÁTH, A., TERBE, ZS., HORVÁTH, E., BORSODI, A., DOBÓ, K., KOVÁCS, E. and KANCSÁR, T. (2000): Duhoux effect on inaperturate gymnosperm and angiosperm pollen grains. - Plant Cell Biology and Development (Szeged) *11*, 128-140.

KEDVES, M. and ROJIK, I. (1993): Biopolymer organization of the exine of *Juniperus virginiana* L., and *Taxus baccata* L. - Plant Cell Biology and Development (Szeged) *4*, 49-63.

KEDVES, M., TÓTH, A. and FARKAS, E. (1991): Effect of the high temperature on the morphological characteristic features of the sporomorphs II. - Acta Biol. Szeged *37*, 25-44.

KEDVES, M. and UNGVÁRI, E. (1996): X-ray effect to the LM morphology of some gymnosperm and angiosperm pollen grains. - Plant Cell Biology and Development (Szeged) *7*, 75-81.

LEJOLY-GABRIEL, M. (1986): Daily and hourly pollen dispersal in Belgium. - 3rd Internat. Conf. on Aerobiology, August 6-9, 1986, Basel, Switzerland, 64.

MIYOSHI, N. (1980): Pollen morphology by means of scanning electron microscope. - Bull. Hiruzen Research Inst. *4-5*, 25-32.

MÜLLER-STOLL, W.R. (1948): Zytomorphologische Studien am Pollen von *Taxus baccata* L. und anderen Koniferen. - Planta *35*, 601-641.

NIKLAS, K.J. (1985): Wind pollination of *Taxus cuspidata*. - Amer. J. Bot. *72*, 1-13.

PEHLIVAN S. (1995): Türkiye'nin alerjen polenleri atlasi. - Matbacilik Sanayi ve Ticaret Ltd. Sirketi,Ankara.

SUÁREZ-CERVERA, M. y SEAONE-CAMBA, J.A. (2001): Estructura y función de la intina de los granos de polen en angiospermas. - Congress on Tropical and Subtropical Palynology (America-Africa), Havanna, Abstracts, 4.

SOUTHWORTH, D. (1974): Solubility of pollen exines. - Amer. J. Bot. *61*, 36-44.

SOUTHWORTH, D. (1986a): Pollen exine substructure. III. *Juniperus communis*. - Can. J. Bot. *64*, 983-987.

SOUTHWORTH, D. (1986b): Isolation of exines from gymnosperm pollen. - Amer. J. Bot. *75*, 15-21.

TAKEOKA, M. (1966): Electronmicroscopic investigations on the surface structure of pollen membrane of trees. - Sci. Repts. Kyoto Pref. Univ. Agriculture *17*, 43-69.

UENO, J. (1974): Some palynological observations of the family tree of Gymnosperms. - Japanese Journal of Palynology *14*, 1-32.

VASIL, V. and SAHNI, R.K. (1964): Morphology and embryology of *Taxodium mucronatum* TENORE. - Phytomorphology *14*, 369-384.

VILLAR, M., KNOX, R.B. and DUMAS, C. (1984): Effective pollination period and nature of pollen-collecting apparatus in the gymnosperm. *Larix leptolepis*. - Ann. Bot. *53*, 279-284.

WODEHOUSE, R. G. (1935): Pollen grains. - McGraw-Hill, New York.

XI YI-ZHEN (1986): Studies on Pollen Morphology of Taxaceae of China. - Acta Phytotaxonomica Sinica 24, 247-252.

6. LM AND TEM INVESTIGATIONS ON PARTIALLY DEGRADED POLLEN GRAINS OF CHENOPODIUM ALBUM

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Abstract

Pollen grains of *Chenopodium album* L. were partially degraded with 2-aminoethanol, $KMnO_4$, and merkaptoethanol. Based on the LM studies, it was established that the molecular system of the sporopollenin of the ectexine of this species is extremely less resistant, and it may be destroyed easily with 2-aminoethanol. Similar experiments on further species of the genus *Chenopodium*, and further taxa of Chenopodiaceae, as well as the pollen of morphologically similar Amaranthaceae, seem to be necessary to better understand the molecular system of the sporopollenin of this kind of periporate pollen grain.

Key words: Palynology, angiosperm, *Chenopodium album*, partial degradation, LM, TEM.

Introduction

The bibliographical data concerning the allergenic character of the pollen grains of the genus *Chenopodium* were summarized by MOLNÁR (1999).

NILSSON, PRAGLOWSKI and NILSSON (1977) investigated with the LM, TEM and SEM method the pollen grains of *Chenopodium album* L. It was established that the pori of the pantoporate pollen grains are covered with operculi. The tectum is perforated, and the surface is covered by spinules.

SEM picture of *Chenopodium ambrosoides* L. was published by HUANG (1998). ERDTMAN (1966) pointed out the morphological similarities between the Chenopodiaceae and the amaranthoid type of the Amaranthaceae. The gomphrenoid type of the Amaranthaceae occurs in the Caryophyllaceae, and is different from the amaranthoid type. The number of the pores of periporate (pantoporate) pollen grains has been used for taxonomic purposes. MCANDREWS and SWANSON (1967) reviewed several methods in this respect and finally they concluded that the C/D ratio is a character of taxonomy, cf. KAPP, DAVIS and KING (2000). In the aeropalynological papers, probably the above mentioned similarities are the reason for the different accuracy in the determinations of this kind of pollen grains. Some selected examples are as follows:

Chenopodium album: DE LEONARDIS et al. (1986), LEBBE, VIGNES and HIDEUX (1988), AGNIHOTRI and SINGH (1991), MEZEI et al. (1992), FERREIRO, RODRIGUEZ and AIRA (2001).

Chenopodium: WANG XIAN-ZENG (1986), SINGH and DEVI (1991), BEN TIBA et al. (1995), PEHLIVAN (1995), MUNUERA GINER et al. (2000).

Chenopodiaceae-Amaranthaceae: AROBBA (1986), CHAUDHARY and SINGH (1991), MAJUMDAR and CHANDA (1991), JÁRAI-KOMLÓDI (1991), ELVIRA RENDUELES et al.

(2000), RUIZ, CANO and DIAZ DE LA GUARDIA (2000), TORTAJADA and MATEU (2000). Following JÁRAI-KOMLÓDI and MEDZIHRADSZKY (1993), "the late summer-autumn season caused first of all by *Ambrosia* and *Artemisia,* and members of the Chenopodiaceae and Amaranthaceae families." (P: 49).

Amaranthaceae/Chenopodiaceae: CHEN and HUANG (1980), NILSSON (1990), MURRAY, SONAGLIONI and VILLAMIL (2001).

The aim of this paper to establish the solubility of the sporopollenin of the ectexine with our standard method with 2-aminoethanol by light and transmission electron microscopical methods and to investigate the qualitative and quantitative morphological alterations in consequence of the mounting media, stain and the embedding processes for TEM studies.

Materials and Methods

The material investigated was collected by Miss K. PRISKIN from Szeged, and by Miss J. SASHALMI from Hódmezővásárhely from weedy associations. The partial degradation was made two times, because at the first result the sporopollenin was extremely less resistant, it was degraded very easily. We believed, that with this fresh material, the polymerization of the sporopollenin is not completely finished, in this way, after three months the experiments with 2-aminoethanol were repeated. After the first set of experiments there are two numbers, the second one is the repeated number. Unstained (a) and stained pollen grains with Safranin T (b) were investigated with the LM method. Temperature 30°C

5 mg pollen + 2 ml 2-aminoethanol, length of time 24 hours, T-12-82, T-12-137.

5 mg pollen + 2 ml 2-aminoethanol, length of time 48 hours, T-12-83, T-12-138.

5 mg pollen + 2 ml 2-aminoethanol, length of time 72 hours, T-12-84, T-12-139.

5 mg pollen + 2 ml 2-aminoethanol, length of time 24 hours washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-85.

5 mg pollen + 2 ml 2-aminoethanol, length of time 48 hours, washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-86.

5 mg pollen + 2 ml 2-aminoethanol, length of time 72 hours, washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-87.

5 mg pollen + 2 ml 2-aminoethanol, length of time 24 hours, washing, + 2 ml merkaptoethanol, length of time 24 hours, T-12-88.

5 mg pollen + 2 ml 2-aminoethanol, length of time 48 hours, washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-89.

5 mg pollen + 2 ml 2-aminoethanol, length of time 72 hours, washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-90

5 mg pollen + 5 ml glycerin 50%, length of time 30 days.

The pollen grains were mounted in glycerine-jelly hydrated to 39.6%. For TEM studies the investigation material was postfixed with OsO_4 aq. dil. 1%, and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made with glass knives on a Porter Blum ultramicrotome. The pictures were taken on a Tesla BS-540 instument (resolution 6-7 Å). All pictures are unretouched.

Results

LM results (Plate 6.1., figs. 1-70)

The fresh pollen grains (Plate 6.1., figs. 1-6) are typically periporate, diameter from 18.0 µm - 33.0µm.

After treatment with 2-aminoethanol during 24 hours, (Plate 6.1., figs. 7-13) the degradation of the ectexine are well shown by the LM method. There are some differences in the degree of the degradation between the first (Plate 6.1., figs. 7,8) and the second experiment (Plate 6.1., figs. 9,10). The ectexine of the pollen grains of the second experiment seems to be a little more resistant than that of the first one. The ectexine of the OsO_4 postfixed and embedded pollen grains in Araldite was more degraded in the first experiment (Plate 6.1., figs. 11,12) than in the second one (Plate 6.1., fig. 13).

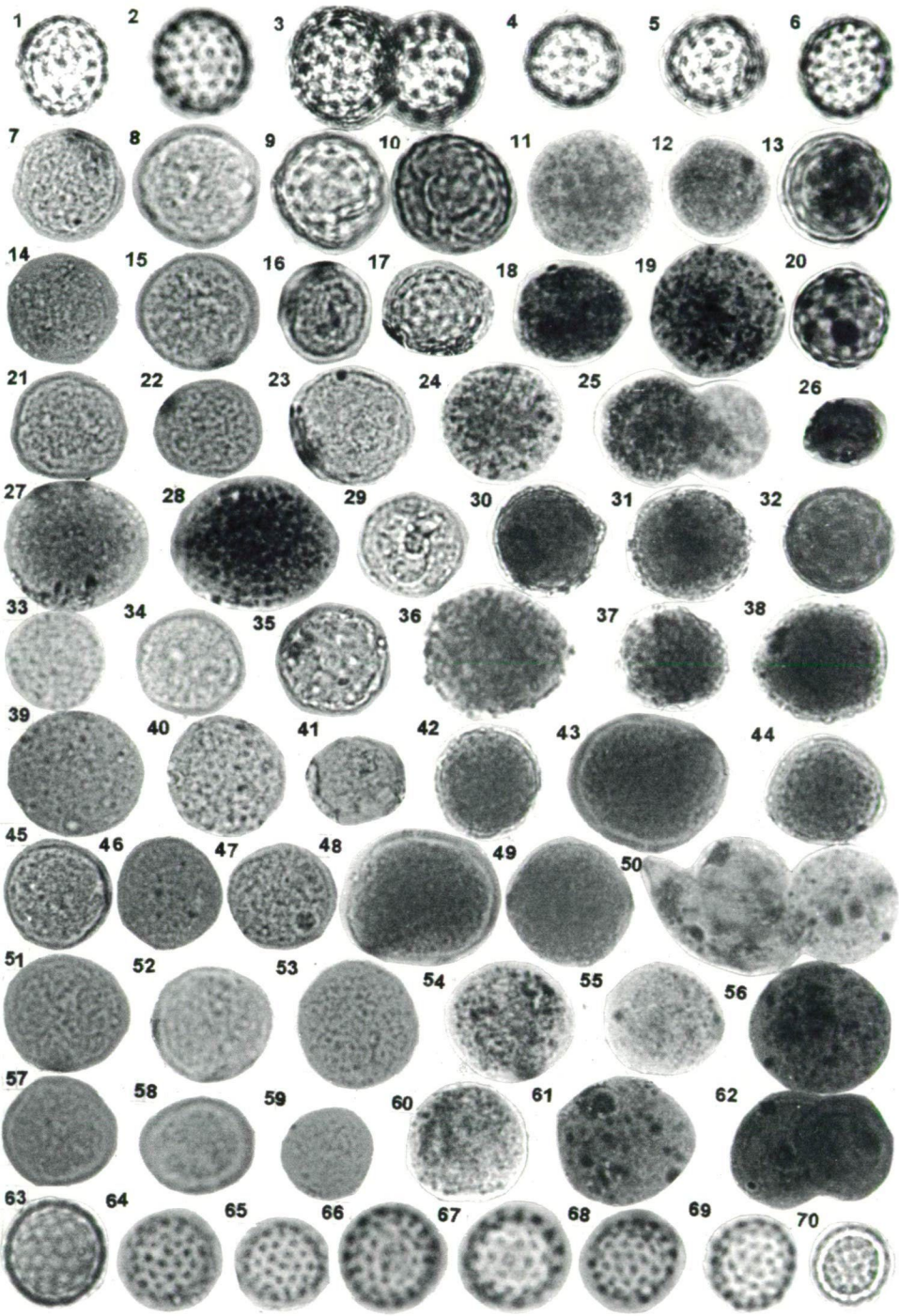
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Plate 6.1.

Plate 6.1.

1-70. *Chenopodium album* L., LM pictures, 750x.
1-6. Fresh pollen grains mounted in glycerine-jelly;
7-10. Pollen grains degraded in 2-aminoethanol during 24 hours, mounted in glycerine-jelly. figs. 7,8. first, 9,10 second experiment.
11-13. Pollen grains degraded in 2-aminoethanol during 24 hours, mounted in Araldite, after embedding processes, figs. 11,12, first, 13, second experiment.
14-17. Pollen grains degraded in 2-aminoethanol during 48 hours, mounted in glycerine-jelly, figs. 14,15 first, 16,17 second experiment.
18-20. Pollen grains degraded in 2-aminoethanol during 48 hours, mounted in Araldite after embedding processes, figs. 18,19 first, 20 second experiment.
21-23. Pollen grains degraded in 2-aminoethanol during 72 hours, mounted in glycerine-jelly, figs. 21,22 first, 23 second experiment.
24-26. Pollen grains degraded in 2-aminoethanol during 72 hours, mounted in Araldite after embedding processes, figs. 24,25 first, 26 second experiment.
27-70. Pollen grains prepared with the first experiment.
27-32. Pollen grains degraded in 2-aminoethanol during 24 hours, washed and oxidized with KMnO4 1% during 24 hours. Figs. 27-29 pollen grains mounted in glycerine-jelly, 30-32 mounted in Araldite after embedding processes.
33-38. Pollen grains degraded in 2-aminoethanol during 48 hours, washed and oxidized with KMnO4 1% during 24 hours. Figs. 33-35 pollen grains mounted in glycerine-jelly, 36-38 mounted in Araldite after embedding processes.
39-44. Pollen grains degraded in 2-aminoethanol during 72 hours, washed and oxidized with KMnO4 1% during 24 hours. Figs. 39-41 pollen grains mounted in glycerine-jelly, 42-44 mounted in Araldite after embedding processes.
45-50. Pollen grains degraded in 2-aminoethanol during 24 hours, washed and partially dissolved with merkaptoethanol during 24 hours. Figs. 45-47 pollen grains mounted in glycerine-jelly, 48-50 mounted in Araldite after embedding processes.
51-56. Pollen grains degraded in 2-aminoethanol during 48 hours, washed and partially degraded with merkaptoethanol during 24 hours. Figs. 51-53 pollen grains mounted in glycerine-jelly, 54-56 mounted in Araldite after embedding processes.
57-62. Pollen grains degraded in 2-aminoethanol during 72 hours, washed and partially degraded with merkaptoethanol during 24 hours. Figs. 57-59 pollen grains mounted in glycerine-jelly, 60-62 mounted in Araldite after embedding processes.
63-70. Pollen grains partially dissolved in glycerine 50% during 30 days, mounted in glycerine-jelly. Magnifications: 750x.

Results after partial degradation with 2-aminoethanol during 48 hours (Plate 6.1., figs. 14-20). The ectexine of the pollen grains of the first experiment mounted in glycerine-jelly (Plate 6.1., figs. 14,15) is hardly damaged. It is well shown that the characteristic periporate character is not perceptible. But on the pollen grains of the second experiment (Plate 6.1., figs. 16,17) there are differences in this respect namely that the polyporate character is more or less preserved. This phenomenon is more characteristic of the pollen grains prepared for TEM studies (Plate 6.1., figs. 18,19), respectively Plate 6.1., fig. 20.

Results after partial degradation with 2-aminoethanol during 72 hours (Plate 6.1., figs. 21-26). The degradation of the sporopollenin is well shown in the pollen grains of the first (Plate 6.1., figs. 21,22) and the second experiment (Plate 6.1., fig. 23) mounted in glycerine-jelly. The pollen grains after partial degradation and embedding processes and mounted in Araldite are similar to the pollen grains mounted in glycerine-jelly, Plate 6.1., figs. 24,25 illustrates the first experiment, Plate 6.1., fig. 26 the pollen grains of the second experiment.

Partial degradation with 2-aminoethanol during 24 hours, washing and oxidizing with $KMnO_4$ 1% during 24 hours (Plate 6.1., figs. 27-32). The degradation of the ectexine is well shown in the pollen grains mounted in glycerine-jelly (Plate 6.1., figs. 27-29) and in Araldite (Plate, 6.1., figs. 30-32). The osmium affinity of the degraded protoplasm is characteristic. Picture 32 on Plate 6.1. illustrate the osmium accumulation around the remnants of the pores.

Partial degradation with 2-aminoethanol during 48 hours, washing and oxidizing with $KMnO_4$ during 24 hours (Plate 6.1., figs. 33-38). The results of this experiment are similar or identical to the previous one.

Partial degradation with 2-aminoethanol during 72 hours, washing and oxidizing with $KMnO_4$ during 24 hours (Plate 6.1., figs. 39-44).

Remnants of the ectexine was observed in these pollen grains. The osmium affinity of the embedded pollen grains was not so intensive than previously (Plate 6.1., figs. 42-44).

Partial degradation with 2-aminoethanol during 24 hours, washing, and continued with merkaptoethanol during 24 hours (Plate 6.1., figs. 45-50)

Characteristic ectexine remnants are in the partially degraded pollen grains mounted in glycerine-jelly (Plate 6.1., figs. 45,47). It is worth mentioning that the remains of the pores were not perceptible. Further strong degradation was observed in the embedded pollen grains (Plate 6.1., figs. 48-50). The measure of the degradation was not completely the same in different specimens. This may be the consequence of several reasons. Near "protoplast" with electron dense granular units in the remnant of the protoplasm is characteristic (Plate 6.1., fig. 50).

Partial degradation with 2-aminoethanol during 48 hours, washing and continued with merkaptoethanol during 24 hours (Plate 6.1., figs. 51-56).

The degraded pollen grains mounted in glycerine-jelly (Plate 6.1., figs. 51-53) are identical to the previous experiment. The ectexine of the embedded pollen grains is nearly destroyed, there are electron dense granular units in the degraded protoplasm (Plate 6.1., figs. 54-56).

Partial degradation with 2-aminoethanol during 72 hours, washing and continued with merkaptoethanol during 24 hours (Plate 6.1., figs. 57-62)

Based on our observations the results of this experiment are identical with the previous one.

Partial dissolution with glycerine (50%) during 30 days (Plate 6.1., figs. 63-70)

The light microscopical morphology of the pollen grains are identical to the fresh untreated specimens.

Quantitative results

Remark. - a = unstained, b = stained with Safranine T, Ar = pollen grains mounted in Araldite.

Nos of experiments	13.0	15.0	18.0	20.0	23.0	25.0	28.0	30.0	33.0	35.0	38.0	40.0µm
T-12-81			0.5	6.0	13.0	26.0	37.5	15.5	1.5.			%
T-12-82a			0.5	12.0	15.0	37.0	20.5	9.0	3.5	1.0	0.5	5.0
T-12-82b			3.5	12.0	12.0	42.5	16.5	10.0	3.5			
T-12-137a		3.0	13.5	15.0	33.0	22.0	8.5	3.5	1.5			
T-12-82Ar	2.5	2.0	5.0	15.0	17.0	26.0	16.5	16.0	8.0	0.5	1.5	

T-12-83a				6.0	12.5	17.5	34.5	19.0	5.5	5.0	
T-12-83b			11.0	18.0	10.0	36.0	21.0	4.0			
T-12-138a			4.5	16.0	19.5	28.0	18.0	11.0	2.5	0.5	
T-12-83Ar			4.0	13.0	14.0	33.0	13.5	11.5	7.5	3.5	
T-12-84a		2.0	9.0	18.5	22.0	31.0	11.5	5.0	0.5	0.5	
T-12-84b		2.0	4.5	16.5	11.0	36.0	18.0	12.0			
T-12-139a		0.5	3.5	14.5	17.0	25.5	18.0	14.0	4.0	3.0	
T-12-84Ar		1.6	3.0	16.0	15.0	33.0	10.0	14.0	5.7	1.7	
T-12-85a		1.5	3.0	6.0	5.5	22.0	15.5	25.5	13.5	6.5	1.0
T-12-85Ar		0.8	0.8	2.2	4.5	15.0	25.4	26.0	14.1	9.0	2.2
T-12-86a		1.0	3.0	13.0	13.0	25.0	18.0	18.0	6.0	3.0	
T-12-86Ar		1.2	4.6	2.3	6.9	20.7	26.4	18.4	9.2	8.0	2.3
T-12-87a			0.5	6.5	6.5	26.5	20.0	23.0	9.5	6.0	1.5
T-12-87Ar			1.0	5.0	12.5	21.5	20.0	19.0	11.5	5.5	4.0
T-12-88a			0.5	10.5	10.0	27.5	22.5	16.0	9.5	3.0	0.5
T-12-88Ar			4.6	20.0	10.8	26.1	13.8	17.0	4.6	3.1	
T-12-89a			0.5	9.0	11.0	23.0	19.0	20.0	12.0	5.0	0.5
T-12-89Ar			5.0	13.0	9.0	27.5	24.0	10.0	8.5	2.5	0.5
T-12-90a			0.5	12.0	11.5	33.5	20.0	16.5	6.0		
T-12-90Ar	1.0	1.5	4.0	13.0	16.5	22.5	15.5	17.0	6.0	3.0	
T-12-91				9.0	44.5	31.0	14.5	1.0			

Based on our results, the smallest pollen grain was 13.0 µm, the largest was 40.0 µm. As regards the quantities of the different sizes it may be established, that this is nearly the same in all experiments, except the partial dissolution with diluted glycerine. The greatest part of the pollen grains are of 25.0 or between 20.0 - 30.0 µm. It is interesting that the pollen grains dissolved in diluted glycerine are from 18.0 to 28.0 µm, so the size range is relatively short, and 44.5% is of 20.0 µm.

TEM results (Plate 6.2., figs.1-8)

Pollen grains from all experiments were embedded and investigated with the transmission electron microscope. As it was established during the light microscopical studies, the ectexine was unusually damaged. Because of the hardly damaged ectexine we present from our documents some selected examples as follows:

Pollen grains treated with 2-aminoethanol during 24 hours (Plate 6.2., figs. 1,2)

The general survey picture illustrates well the more or less homogenized protoplasm, and the electron dense and extremely damaged ectexine (Plate 6.2., fig. 1). The foot layer is not completely destroyed, fragments of the infratectal layer are also present. In the highly magnified picture (Plate 6.2., fig. 2) the outer part of the foot layer is granular, which may be the biopolymer units of the ectexine. The inner part is more or less homogeneous.

Pollen grains treated with 2-aminoethanol during 48 hours (Plate 6.2., fig. 3)

Damaged protoplasm and the fragment of the foot layer are illustrated. Between the remnant of the plasma membrane and the foot layer there is a light zone.

Pollen grains treated with 2-aminoethanol during 72 hours (Plate 6.2., fig. 4-6)

Ultrastructure of ectexine lost "inner body" is illustrated in fig. 4, Plate 6.2. The protoplasm is after treatment homogeneous the organelles were degraded. The ultrastructure of the ectexine remnants (Plate 6.2., figs. 5,6) is granular, and there are more or

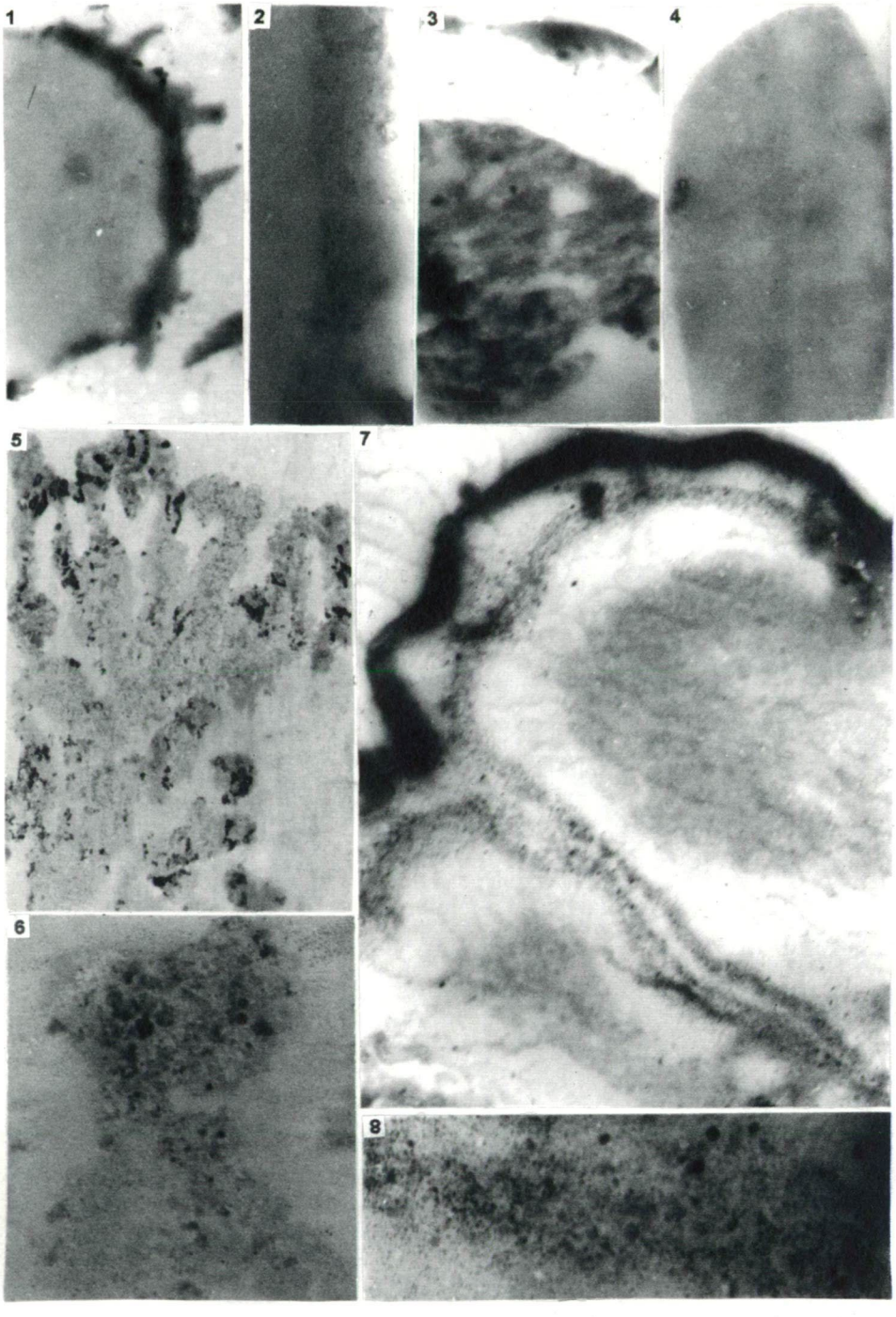

Plate 6.2.

Plate 6.2.

Chenopodium album L. TEM pictures.

1. General survey picture from the partially degraded pollen grain (T-12-82), Negative No.: 8445, 15.000x.
2. Detail from the partially degraded inner layer of the ectexine (foot layer). The globular elements of the biopolymer structure are well shown. (T-12-82), Negative No.: 8446, 75.000x.
3. Degraded wall and protoplasm with experiment T-12-83, Negative No.: 8451, 15.000x.
4. Degraded "inner body" with experiment T-12-84, Negative No.: 8448.
5. Detail from the damaged ectexine, experiment T-12-84, Negative No.: 8490, 15.000x.
6. Highly magnified picture from the degraded ectexine. The globular biopolymer units are well shown. T-12-84, Negative No.: 8492, 75.000x.
7. General survey picture from the partially degraded pollen grain. T-12-85, Negative No.: 8452, 15.000x.
8. Detail from the biopolymer units of the partially degraded ectexine. T-12-85. Negative No.: 8451, 75.000x.

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less globular electron dense units. The large units may be elements of the biopolymer system of the quasi-periodic arrangement.

Pollen grains treated with 2-aminoethanol during 24 hours, washing and oxidized with $KMnO_4$ during 24 hours (Plate 6.2., figs. 7,8)

Damaged protoplasm a light zone and a granular layer, followed by an electron dense outer layer is illustrated in picture 7, Plate 6.2. The granular layer may be the endexine or the inner part of the foot layer. In the highly magnified picture (Plate 6.2., fig. 8) the ultrastructure of this inner layer is granular with electron dense globular units, similar to the previous ultrastructure (Plate 6.2., fig. 6).

Discussion and Conclusions

1. The organization of the biopolymer system of the spore-pollen wall, based on the newest results, is more complicated than was believed earlier. It is well established that this question may not be resolved with one model. The composition change in the different taxa, the different part of the ectexine within one species and several further factors.

SOUTHWORTH (1974) established that the old pollen of dicots dissolves readily in 2-aminoethanol. During our different kind of experiments we established that the walls of some sporomorphs are easily soluble in organic solvents. As a good example, the ectexine of *Quercus* and the exospore of the spores of *Equisetum*, may be mentioned, cf. KEDVES and GÁSPÁR (1994, 1996). KEDVES et al. (1998) established that the pollen grains of *Platanus hybrida* BROT. and *Tilia platyphyllos* SCOP. dissolved easily in diethylamine, but were resistant to merkaptoethanol and further alcohols (methanol, ethanol, n-propanol, n-pentanol and i-amyl alcohol). These new experiments verified again the extremely heterogeneous and dynamic character of the molecular system of the sporopollenin.

2. It may be emphasized again, the extremely multifaceted aspect of the study of the molecular and highly organized biopolymer system of the spore-pollen wall. Ecological factors are extremely important. The molecular transformation of the sporopollenin may be taken into consideration in the evaluations of the experimental results.

3. In the polyporate pollen grains, the resistance of the pollen grains of the *Juglans* genus may be emphasized (KEDVES and KINCSEK, 1989, KEDVES, KÁROSSY and BORBOLA, 1997). In this way, the polyporate morphological character may not be the first factor of the less resistant ectexine of the genus *Chenopodium.*

4. In the future, control investigations will be necessary. In this respect, the partial dissolution of the pollen grains further species of the genus *Chenopodium,* and different taxa of the Chenopodiaceae and Amaranthaceae will be important.

Acknowledgements

This work was supported by Grant OTKA T 031715. The writers are deeply indebted to Dr. J.F. LAING Senior Palynologist (Robertson Research International Ltd. Llandudno, Gwynedd, U.K.) for critically reviewing the manuscript and for his valuable comments.

References

AGNIHOTRI, M.S. and SINGH, S. (1991): Allergenicity of some common airborne pollen of Indo-Gangetic plain and their role in oral hyposensitization in patients of allergic diseases. - 6th National Conf. on Aerobiology, Abstracts, 17.

ANDERSEN, S. TH. (1980): Influence of climatic variation on pollen season severity in wind-pollinated trees and herbs. - Grana *19*, 47-52.

AROBBA, D. (1986): Modern pollen deposition in Genoa area (Italy). A first contribution to the drawing of palynological maps. - 3rd Internat. Conf. on Aerobiology, Abstracts, 70.

BEN TIBA, B., TABKA, Z., KHELIFA, S., DAVID, B., ZEBIDI, A.et VASSEUR, B. (1995): Calendrier pollinique de la ville de Sousse (Tunisie) résultats préliminaires Polinic calendar of the Soussa city (Tunisia). Preliminary results. - 2nd Symposium on African Palynology, Tervuren (Belgium) Publ. Occas. CIFEG 1995/31, Orléans, 173-181.

BOEHM, G. et LEUSCHNER, R.M. (1989): Les polluants aériens à diverses altitudes . - Rev. Internat de Pédiatrie *193,* 26-33.

CHAUDHARY, R.P. and SINGH, R.L. (1991): Aeropalynological survey, pollen and fungal spores, in Kathmandu valley (Central Himalaya). - 6th National Conf. on Aerobiology, Abstracts, 52-53.

CHEN, S.-H. and HUANG, T.-c. (1980): Aeropalynological study of Taipei Basin, Taiwan. - Grana *19*, 147-155.

DE LEONARDIS, W., LONGHITANO, N., MELI, R., PICCIONE, V., ZIZZA, A., CRIMI, N., PALERMO, F. and MISTRETTA, A. (1986): Flora of allergenic pollen grains in Italy. - 3rd Internat. Conf. on Aerobiology, Abstracts, 70.

ELVIRA RENDUELES, B., MORENO GRAU, S., ANGOSTO, J.M., BAYO, J., MORENO, J.M. y MORENO CLAVEL, J. (2000): Evolución de los taxones Urticaceae, Cupressaceae, Chenopodiaceae-Amaranthaceae, *Olea,* Poaceae y *Zygophyllum* en 6 años de muestre aerobiologico en Cartagena (España) 1993/99. - Libro de Resumenes XIII Simposio de la Asociación de Palinólogos en Lengua Española (A.P.L.E.), 43-44.

ERDTMAN, G. (1966): Pollen morphology and plant taxonomy - angiosperms (An Introduction to Palynology. I). - Hafner Publ. Comp., New York and London.

FERREIRO, M., RODRIGUEZ, F.J. y AIRA, M.J. (2001): Estudio comparativo de datos clinicos y aerobiologicos en la ciudad de A Coruña. - Congress on Tropical and Subtropical Palynology (America-Africa), Abstracts, 44.

GARCIA-MOZO, H., GALÁN-SOLDEVILLA, C. y DOMINGUEZ-VILCHES, E. (2000): Calendario polinico del parque natural de la Sierra de Hornachuelos (Córdoba), 1998-2000. - Libro de Resumenes XIII Simposio de la Asociación de Palinólogos en Lengua Española (A.P.L.E.), 31-32.

HUANG, T.-C. (1998): Airborne Pollen Grains and Spores in Taiwan. - Taipei.

JÁRAI-KOMLÓDI, M. (1991): First results of a study on airborne sporomorphs in Budapest, Hungary. - Grana *30*, 464-466.

JÁRAI-KOMLÓDI, M. and MEDZIHRADSZKY, ZS. (1993): Aeropollen research in the air of Budapest in 1992. - Studia Bot. Hungarica *24*, 45-49.

KAPP, R.O., DAVIS, O.K. and KING, J.E. (2000): Ronald O. Kapp's Pollen and Spores Second Edition. - AASP Foundation, Texas, U.S.A.

KEDVES, M. and GÁSPÁR, I. (1994): Les altérations sécondaires des spores et des grains de pollen dissous partiellement. - Trabajos de palinologia básica y aplicada. Eds.: Isabel MATEU ANDRÉS, Michele

DUPRÉ, OLLIVIER Jaime GUEMES HÉRAS y Maria ELVIRA BURGAZ MORENO Universitad de Valencia, 153-161.

KEDVES, M. and GÁSPÁR, I. (1996): New data concerning the solubility of the pollen grains of the genus Quercus L. - Plant Cell Biology and Development (Szeged) *7*, 56-61.

KEDVES, M., HORVÁTH, E., MÉSZÁROS, E., MÉSZÁROS, R., RONTÓ, G., SZLÁVIK, N., GAUDÉNYI, SZ., and KALMÁR, Á. (1998): LM investigations of partially dissolved sporomorphs II. - Plant Cell Biology and Development (Szeged) *9*, 76-87.

KEDVES, M., KÁROSSY, Á. and BORBOLA, A. (1997): LM investigations of partially dissolved sporomorphs I. - Plant Cell Biology and Development (Szeged) *8*, 44-55.

KEDVES, M. and KINCSEK, I. (1989): Effect of the high temperature on the morphological characteristic features of the sporomorphs I. - Acta Biol. Szeged. *35*, 333-335.

LEBBE, J., VIGNES, R. et HIDEUX, M. (1988): Base de connaissances sur les pollens et spores atmosphériques de l'Europe du Nord et tempérée : identification et applications taxonomiques. - Ann. Sci. Nat. Not., Paris, *13* ser, 9, 107-123.

LEUSCHNER, R.M. (1989): 20 Jahre Luft-pollenbestimmung. - DIA-GM *4*, 367-376.

MAJUMDAR, M.R. and CHANDA, S. (1991): Aeropalynology of Cooch Behar town, West Bengal, India. - 6th National Conf. on Aerobiology, Abstracts, 85.

MCANDREWS, J.H. and SWANSON, A.R. (1967): The pore number of periporate pollen with special references to Chenopodium. - Rev. Palaeobot. Palynol. *3*, 105-117.

MEZEI, G., KOMLÓDI, J.M., PAPP, E. and CSERHÁTI, E. (1992): Late summer pollen and allergen spectrum in children with allergic rhinitis and asthma in Budapest. - Pädiatrie und Pädologie 27, 75.

MOLNÁR, T. (1999): A levegőben található pollenek morfológiai vizsgálata és dinamikája. - Debreceni Agrártudományi Egyetem, Mezőgazdaságtudományi Kar, Doktori (Ph.D.) Értekezés.

MUNUERA GINER, M., CARRIÓN GARCIA, J.S., NAVARRO CAMACHO, C., GARCIA SELLÉS, J. y. ESPIN GEA, A. (2000): Propuesta de nuevos tipos polinicos a incluir en pruebas cutáneas de alergias en la región de Murcia. - Libro de Resumenes XIII Simposio de la Asociación de palinólogos en Lengua Española (A.P.L.E.), 55-56.

MURRAY, M.G., SONAGLIONI, M.I. y VILLAMIL, C.B. (2001): Variacion anual del polen en la atmosfera de Bahia Blanca - Argentina. - Congress on Tropical and Subtropical Palynology (America-Africa) Abstracts, 49.

NILSSON, S. (1990): Regional and global distribution of aeroallergens. - Rev. Palaeobot. Palynol. *64*, 29-34.

NILSSON, S., PRAGLOWSKI, J. and NILSSON, L. (1977): Atlas of Airborne Pollen Grains and Spores in Northern Europe. - Natur och Kultur, Stockholm.

PEHLIVAN, S. (1995): Türkiye'nin alerjen pollenleri atlasi. - Matbaacilik Sanayi ve Ticaret Ltd. Sirketi, Ankara.

RICHARD, H., BIDAULT, M., KADMIRI, S., SIMON-RIGAUD, M.L., BOTELLA, N., CHAMBAUD, R., et ROUGEOT, J.C. (1986): Calendrier de la pluie pollinique à Besançon en 1984. - Publication du Laboratoire de Chrono-Ecologie, URA n° 35 du CNRS, Faculté des Sciences, Besançon.

RUIZ, L., CANO, E. y DIAZ DE LA GUARDIA, C. (2000): Calendario polínico de la ciudad de Jaén (Sureste Peninsular): Evolución y estacionalidad del espectro polinico atmosférico. - Libro de Resumenes XIII Simposio de la Asociación de Palinólogos en Lengua Española (A.P.L.E.), 59-60.

SABARIEGO, S., DIAZ DE LA GUARDIA, C., MOTA J.F. y ALBA, F. (2000): Principales tipos polínicos herbáceos en la atmósfera de Almería. - Libro de resumenes, XIII Simposio de la Asociación de palinólogos en Lengua Española (A.P.L.E.), 49.

SINGH, A. B. (1986): Airborne pollen types of allergenic significance to India. - 3rd Internat. Conf. on Aerobiology, Abstracts, 87.

SINGH, N.I. and DEVI, K.K. (1991): Aerobiology and allergenic human diseases in Manipur, II. Airborne pollen grains of Imphal, Imphal, district. - 6th National Conf. on Aerobiology, Abstracts, 145.

SOUTHWORTH, D. (1974): Solubility of pollen exines. - Amer. J. Bot. *61*, 36-44.

TORTAJADA, B. y MATEU, I. (2000): Dos años de estudio aerobiológico de la atmósfera de Valencia. - Libro de Resumenes XIII Simposio de la Asociación de Palinólogos en Lengua Española (A.P.L.E.), 67.

WANG XIAN-ZENG (1986): Pollen Environment Pollinosis. - 3rd Internat. Conf. on Aerobiology, Abstracts, 90.

7. TRANSMISSION ELECTRON MICROSCOPY OF PARTIALLY DEGRADED POLLEN GRAINS OF AMBROSIA ARTEMISIIFOLIA (RAGWEED)

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Abstract

Pollen grains of *Ambrosia artemisiifolia* L. were partially degraded with 2-aminoethanol, potassium permanganate and merkaptoethanol. The partial degradation with 2-aminoethanol and potassium permanganate revealed the biopolymer structure of the ectexine. Several pecularities were established after partial degradation, such as: 1. The very resistant pollenkitt in the holes of the infratectal layer. 2. Rarely micro-organisms were observed on the surface of the tectum. 3. The partial degradation resulted in different layers in the molecular structure of both tectal and infratectal surfaces. 4. The outer part has strong electron density and probably with radially oriented helical structures. 5. The lamellar structure of the foot layer and in particular of the ectexine was also discovered by the combined partial degradation with 2-aminoethanol and potassium permanganate. 6. Network of mostly cyclic molecules were observed at the ultrathin sections of the partially degraded ectexine. 7. The merkaptoethanol at the end revealed the organelles of the protoplasm.

Key words: Palynology, recent, *Ambrosia artemisiifolia*, experimentally degraded, TEM.

Introduction

Pollen grains of *Ambrosia artemisiifolia* are extremely allergenic. This is the reason why a great number of papers are dealing with the presence of the pollen grains of *Ambrosia* in the air. Some selected papers are as follows: GRATER and STEMEN (1967) emphasized, that among the aeroallergenic pollen grains the ragweed have been the most intensively investigated among the aeroallergen palynomorphs. According to O'ROURKE (1996) the *Ambrosia* with 45 species is the most important aeroallergen in North America. Two species (*A. trifida, A. elatior*) was discussed in the first place with the two acidic proteins as major antigens (E and K). LEUSCHNER (1985) pointed, that the *Ambrosia* was discovered in Switzerland in 1970. Later, LEUSCHNER, BOEHM and MARI (1990) discussed the spreading of this plant. It is worth mentioning, that the pollen grains of *Ambrosia* were not included in the monograph of NILSSON, PRAGLOWSKI and NILSSON (1977) and in the atlas of PEHLIVAN (1995). The localisation of the antigenic and allergenic proteins in the intine was established by several authors: KNOX and HESLOP-HARRISON (1970, 1971), KNOX, HESLOP-HARRISON and REED (1970), KNOX, WILLING and ASHFORD (1972). During our TEM studies of partially dissolved pollen grains of ragweed pollen grains chloroplasts were observed in the intine (KEDVES and PÁRDUTZ, 2000). On the surface of the thylakoid membranes different kinds of molecu-

SZTE Egyetemi Könyvtár SZEGED 6.

lar systems were observed which may be important in the biosynthesis of the pollen grains. In another paper (KEDVES, PÁRDUTZ and MADARÁSZ, 2000) a poorly preserved regular pentagon of the partially degraded exine was studied with the symmetry operation, and for the first time the molecular structure of the globular units of the metastable biopolymer system was published. These units are composed of a cluster of cyclic molecules. The occurrence of ragweed pollen grains in Hungary was pointed out by JÁRAI-KOMLÓDI (1991), MEZEI et al. (1991, 1992), JÁRAI-KOMLÓDI and JUHÁSZ (1993), JÁRAI-KOMLÓDI and MEDZIHRADSZKY (1993) and MOLNÁR (1999).

In our experimental studies on recent pollen grains allergenic pollen grains were also investigated. Several times we noticed the presence of micro-organisms on the surface or in the holes of the infratectal layer ectexine which may be factors of combined allergenic effect. The importance of the exine ultrastructure in the allergenic effect was pointed out in several papers. NILSSON, PRAGLOWSKI and NILSSON (1977) published several important TEM data from allergenic pollen grains and spores from Northern Europe. CERCEAU-LARRIVAL (1986), ABADIE et al. (1986, 1988), CERCEAU-LARRIVAL and DEROUET (1988), CERCEAU et al. (1991) emphasized that the channels in the tectum of the pollen grains promote the diffusion of the water soluble allergenic proteins. Ultrastructure of acetolyzed pollen grains of the genus *Ambrosia* were published by PAYNE and SKVARLA (1970).

The aim of this paper is to present in detail the results of the partially degraded pollen grains of *Ambrosia artemisiifolia*, to get further ultrastructural on biopolymer characteristic features, which may be connected to the allergenic character of the pollen grain.

Materials and Methods

The material for this investigation was collected by M. KEDVES on the 1998. The partial degradation was as follows:

Temperature 30°C, 5 mg dry pollen grains.

Experiment No.:1/7-1391. - 1 ml 2-aminoethanol, length of time 24h.

Experiment No.: 1/7-1392. - 1 ml 2-aminoethanol, length of time 48h.

Experiment No.: 1/7-1393. - 1 ml 2-aminoethanol, length of time 72h.

Experiment No.: 1/7-1394. - 1 ml 2-aminoethanol, length of time 24h, washing, + 10 ml 0.01% $KMnO_4$, length of time 24h.

Experiment No.: 1/7-1395. - 1 ml 2-aminoethanol, length of time 48h, washing, + 10 ml 0.01% $KMnO_4$, length of time 24h.

Experiment No.: 1/7-1396. - 1 ml 2-aminoethanol, length of time 72h, washing, + 10 ml 0.01% $KMnO_4$, length of time 24h.

Experiment No.: 1/7-1397. - 1 ml 2-aminoethanol, length of time 24h, washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment No.: 1/7-1398. - 1 ml 2-aminoethanol, length of time 48h, washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment No.: 1/7-1399. - 1 ml 2-aminoethanol, length of time 72h, washing, + 1 ml merkaptoethanol, length of time 24h.

After washing, the pollen material was postfixed with OsO_4 aq. dil. 1% and embedded in Araldite. The ultrathin sections were made in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences on a Porter Blum ultramicrotome. The pictures were taken in a Zeiss EM-902 by resolution 2-3 Å as in Plate 7.2, figs. 1-7, Plate 7.3., figs. 1-4 and a Tesla BS-540, resolution of 6-7 Å, in Plate 7.1., figs. 1-6, and Plate 7.4., figs. 1-6. All pictures are unretouched.

Results

Partial degradation with 2-aminoethanol

Experiment No.: 1/7-1391 (Plate 7.1., figs. 1,2). - The endexine is well separated from the foot layer, its ultrastructure is more or less finely lamellar. Essentially no important alterations were observed. Plasma membrane and the organelles of the protoplasm are relatively well preserved.

Experiment No.: 1/7-1392 (Plate 7.1., fig. 3). - The ultrastructure is similar to the previous sample, differences in the electron density of the ectexine and endexine are observed, namely the ectexine is more electron dense than the endexine.

Experiment No.: 1/7-1393 (Plate 7.1., figs. 4-6). - One microorganism probably of bacterial origin was observed on the surface (Plate 7.1., fig. 4). Characteristic electron dense material of pollenkitt are in the holes of the infratectal layer. The intine separates from the foot layer by its electron density. The degradation of the plasma membrane and the organelles of the protoplasm are characteristic.

Partial degradation with 2-aminoethanol and $KMnO_4$ aq. dil.

Experiment No.: 1/7-1394 (Plate 7.2., figs. 1-7). - This experiment resulted in important alterations in the fine structure of the exine of the pollen grains. Three layers can be distinguished at the originally homogeneous ectexine. The outer and the inner surfaces are characteristic. The outermost electron dense layer (Plate 7.2., figs. 2,3) is composed of more or less globular units. These units are arranged in radially oriented linear structures or their disposition is irregular. A light zone of 28-36 Å follow this layer. The inner part of the ectexine is finely granular (Plate 7.2., figs. 1,2,4,5). These granular structures represent the molecular structures of the ectexine (Plate 7.2., figs. 6,7). The ultrastructure of the inner surfaces of the infratectal layer is identical with the outermost part of the tectum. The partially degraded foot layer is lamellar, with some electron dense particles or layers. The endexine is also lamellar (Plate 7.2., fig. 1). The biopolymer system is well documented with this experiment. The diameter of the globular units are 4-5 Å. There are different kinds of arrangements. Linear, pentagonal (cyclic) and irregular structures were observed (Plate 7.2., fig. 6). The molecular structure sensu strictu (Plate 7.2., fig. 7) represents a network composed of cyclic molecular structures.

Experiment No.: 1/7-1395 (Plate 7.3., figs. 1-4). - The stratification of the ectexine is similar to the previous experiment, but the degradation of the outermost electron dense layer of the ectexine in some parts of the surface is characteristic (Plate 7.3., fig. 1). The ultrastructure of the inner walls of the ectexine is not always the same, in all probability in consequence of the differences in the experimental effect. The lamellar ultrastructure of the foot layer is not so characteristic (Plate 7.3., figs. 1,3), but very characteristic finely lamellar ultrastructure was observed in some parts of the intine (Plate 7.3., fig. 1). The biopolymer and the molecular system of the foot layer is illustrated in fig. 4, Plate 7.3. The molecular structure revealed is also mostly composed of cyclic units. The diameters of the higly organized biopolymer structures are 6-8 Å. Several linear arrangements of these units were observed in more or less radial orientation.

Experiment No.: 1/7-1396 (Plate 7.4., fig. 1). - A general survey picture illustrated the ultrastructural alterations of the ectexine. But the electron density of the endexine is stronger than those of the ectexine including the foot layer too. Fine structure of the intine was not observed. The characteristic degradation of the plasma membrane is illustrated. The organelles of the protoplasm are also partially degraded.

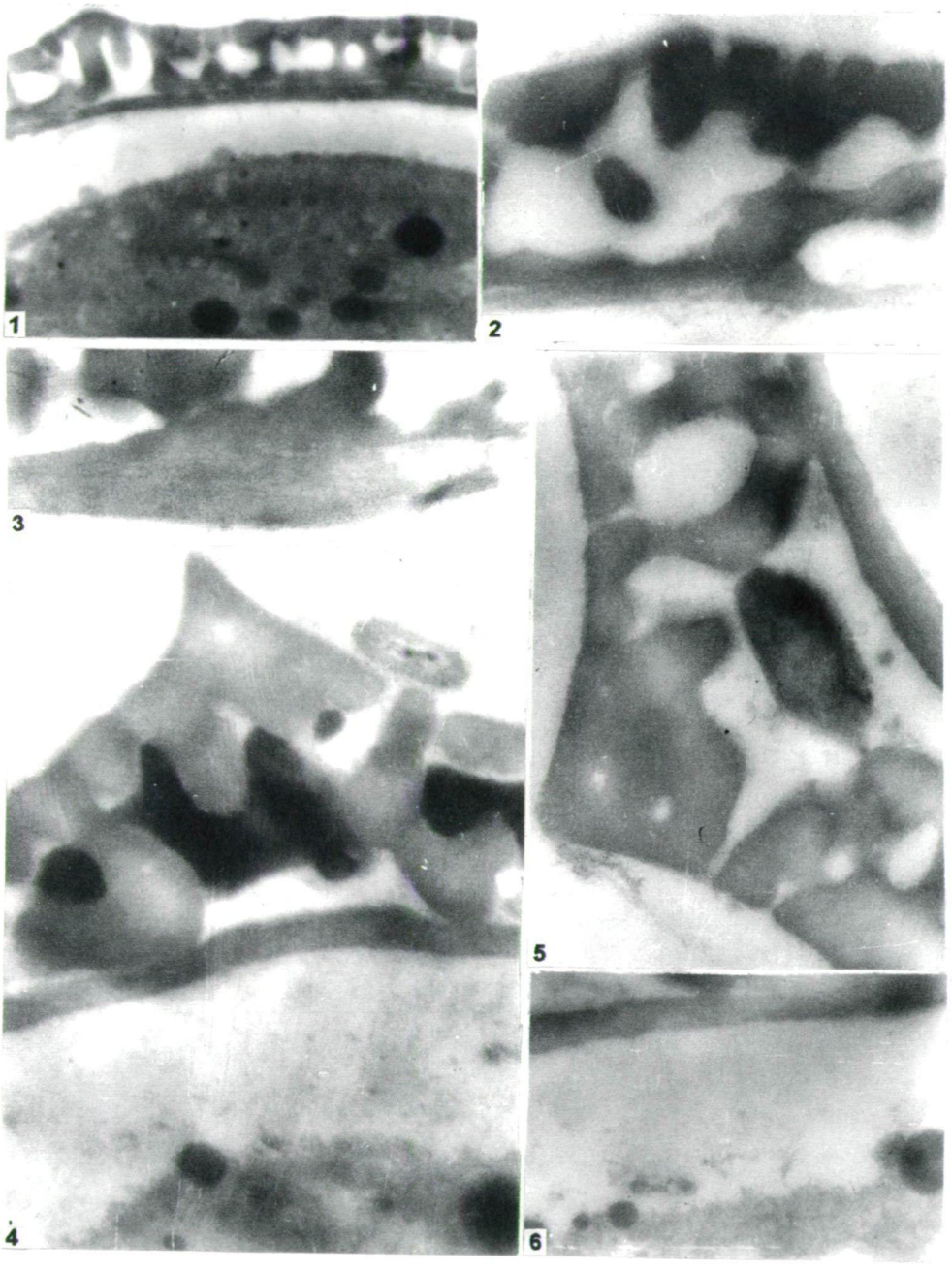

Plate 7.1.

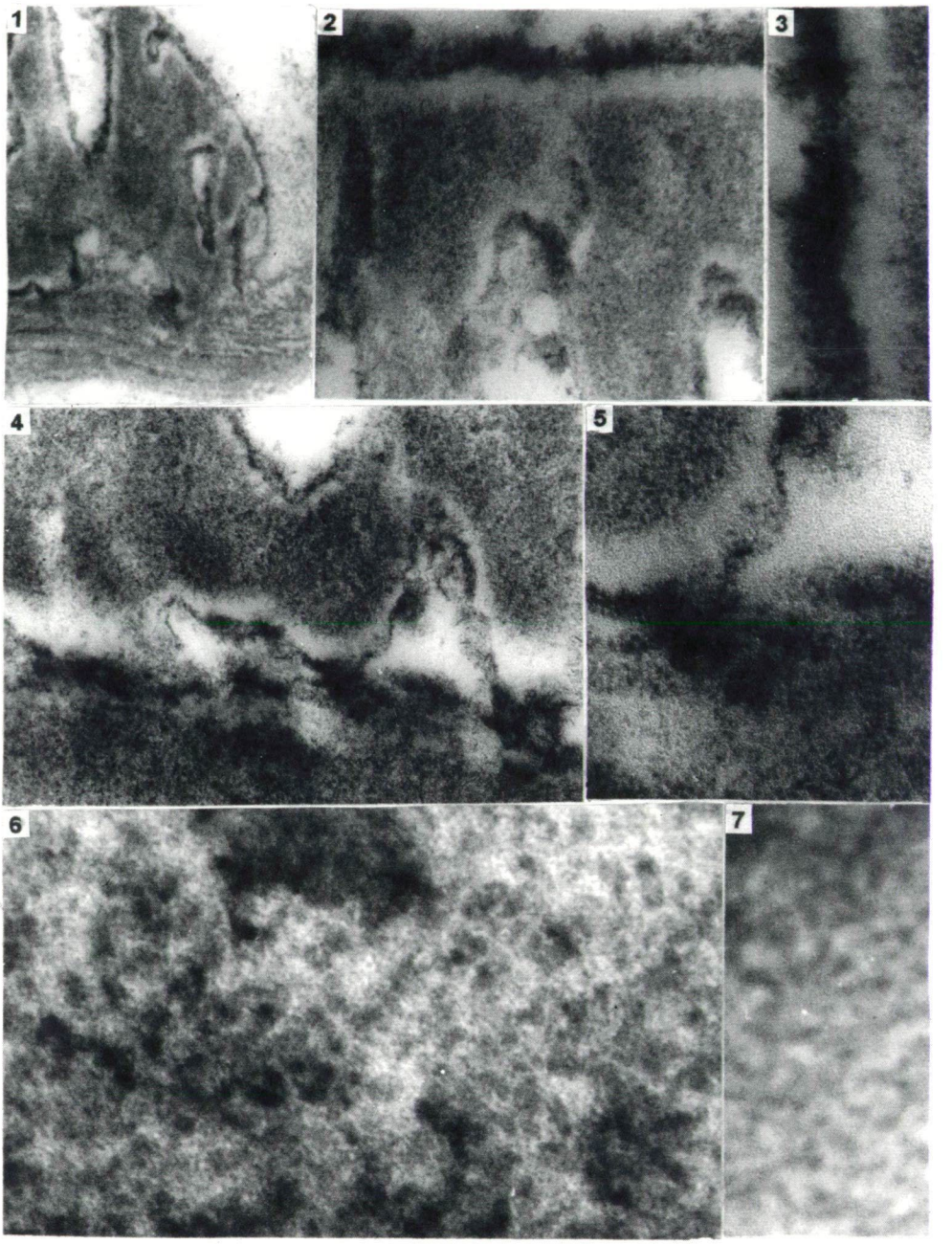

Plate 7.2.

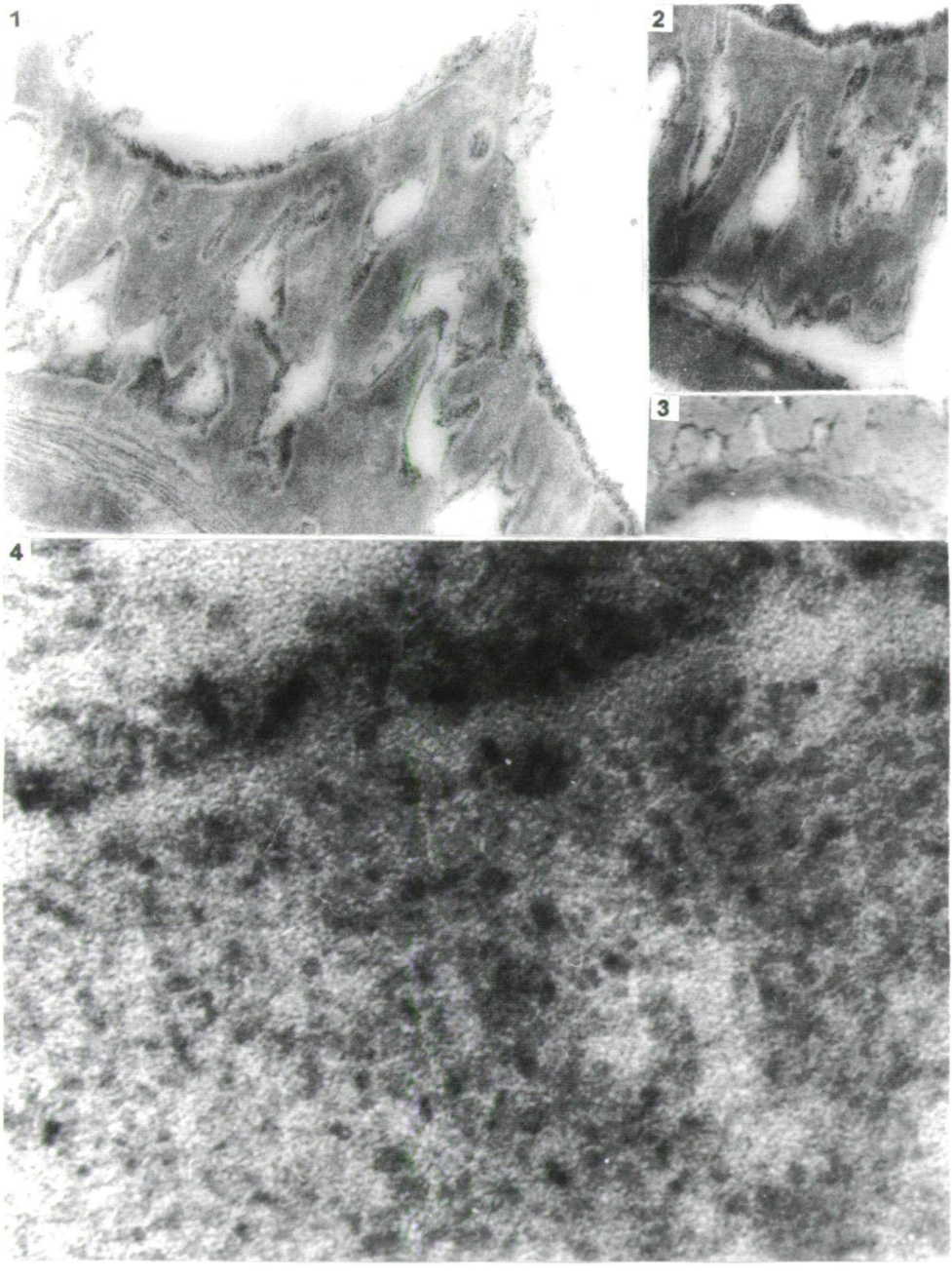

Plate 7.3.

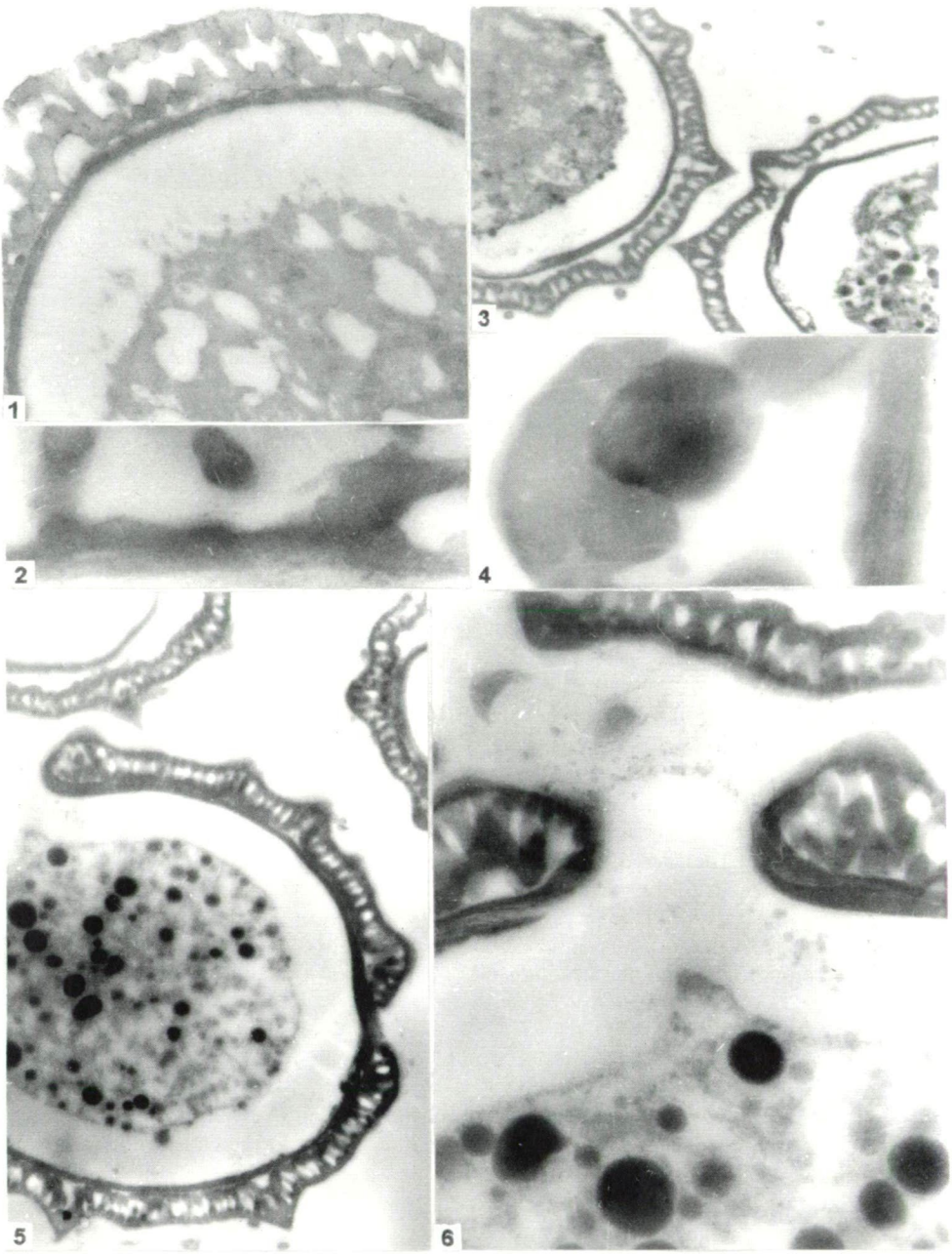

Plate 7.4.

Plate 7.1.

1-6. *Ambrosia artemisiifolia* L. 1. Experiment No.: 1/7-1391, Negative No.: 7385, 12.300x. 2. Experiment No.: 1/7-1391, Negative No.: 7370, 41.000x, 3. Experiment No.: 1/7-1392, Negative No.: 7332, 41.000x. 4. Experiment No.: 1/7-1393, Negative No.: 7338, 41.000x. 5. Experiment No.: 1/7-1393, Negative No.: 7335, 41.000x. 6. Experiment No.: 1/7-1393, Negative No.: 7336, 41.000x.

Plate 7.2.

1-7. *Ambrosia artemisiifolia* L. 1. Experiment No.: 1/7-1394, Negative No.: 7353, 41.000x. 2. Experiment No.: 1/7-1394, Negative No.: 7557, 123.000x. 3. Experiment No.: 1/7-1394, Negative No.: 7558, 205.000x. 4. Experiment No.: 1/7-1394, Negative No.: 7561, 123.000x. 5. Experiment No.: 1/7-1394, Negative No.: 7562, 205.000x. 6. Experiment No.: 1/7-1394, Negative No.: 7563, 820.000x. 7. Experiment No.: 1/7-1394, Negative No.: 7564, 2,050.000x.

Plate 7.3.

1-4. *Ambrosia artemisiifolia* L. 1. Experiment No.: 1/7-1395, Negative No.: 7358, 41.000x. 2. Experiment No.: 1/7-1395, Negative No.: 7357, 41.000x. 3. Experiment No.: 1/7-1395, Negative No.:7401, 41.000x. 4. Experiment No.: 1/7-1395, Negative No.: 7571, 820.000x.

Plate 7.4.

1-6. *Ambrosia artemisiifolia* L. 1. Experiment No.: 1/7-1396, Negative No.: 7380, 12.300x. 2. Experiment No.: 1/7-1397, Negative No.: 7370, 41.000x. 3. Experiment No.: 1/7-1398, Negative No.: 7377, 4.100x. 4. Experiment No.: 1/7-1398, Negative No.: 7373, 41.000x. 5. Experiment No.: 1/7-1399, Negative No.: 7378, 4.100x. 6. Experiment No.: 1/7-1399, Negative No.: 7380, 12.300x.

Partial degradation with 2-aminoethanol and merkaptoethanol

Experiment No.: 1/7-1397 (Plate 7.4., fig. 2). - A remarkable degradation of the intine was observed. The electron dense particles are well presented.

Experiment No.: 1/7-1398 (Plate 7.4., figs. 3-5). - The endexine separates sometimes from the foot layer. Degradation of the intine, sometimes together with the foot layer, was observed. The electron dense particles are present in the holes of the infratectal layer. The intine and the plasma membrane is partially degraded. The protoplasm is full of electron dense particles and light areas, probably with vacuoles.

Experiment No.: 1/7-1399 (Plate 7.4., fig. 6). - The degradation of the plasma membrane is well illustrated together with the different kinds of organelles of the protoplasm. The degradation of the intine is remarkable, but in the apertural area a not so well preserved operculum-like granular structure was observed. The tectum is sometimes dissoluted.

Discussion and Conclusions

Based on our present results, the following may be pointed out:

1. The resistant electron dense particles in the holes of the infratectal layer can be destroyed only by oxidation after the dissolution with 2-aminoethanol.

2. Micro-organisms occur rarely on the perforated tectum, which may be the consequence of the aromatic derivates of the whole plant.

3. The degradation with 2-aminoethanol combined with oxidizing agents discovered several structures of different level of organization of the ectexine.

3.1. On the surface of the tectum and on the inner surfaces also an electron dense layer is present. On the tectum this layer may be composed of radially oriented helical structures. After this layer there is a light zone this is completely new in comparison to the earlier investigated exines.

3.2. The inner part of the tectum and the infratectal layer is of granular structure after partial degradation, namely the biopolymer structures of different organization are well demonstrated.

3.3. The endexine and sometimes the foot layer is finely lamellar after this kind of degradation process.

In comparison with the previous similar degradation experiment it may be emphasized, that there are differences to the previous ones. Namely the infratectal layer was degraded in the first place. The superficial electron dense layer was observed until now in *Phoenix sylvestris* only (cf. KEDVES, BORBOLA, TRIPATHI and KUMAR, 2000).

The ultrastructural data both non-experimental and experimental are useful to understand the allergenic effect of the spores and pollen grains.

Acknowledgements

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References

ABADIE, M., BURY, E. et HIDEUX, M. (1986): Dynamique des glycoprotéides et rôle des microcanaux au cours du développement des microspores et du pollen de *Dactylis glomerata* L.: étude ultrastructurale. In: French-Swedish symposium on pollens of cockfoot (*Dactylis glomerata* L.) and their environment. Stockholm, septembre 17-19, 33-39.

ABADIE, M., HIDEUX, M. et BURY, E. (1988): Détection immunocytochimique et ultrastructurale d'antigènes chez le pollen de *Dactylis glomerata* L. - Ann. Sci. Nat. Bot., Paris *13* ser. *9*, 209-223.

CERCEAU-LARRIVAL, M.-T. (1986): Reserches biopalynologiques sur *Dactylis glomerata* L. French-Swedish Symposium on pollens of Cookfoot (*Dactylis glomerata* L.) and their environment. Stockholm, Sept. 1986, AFSR, report *51*, 13-18.

CERCEAU-LARRIVAL, M.-T. et DEROUET, L. (1988): Relation possible entre les éléments inorganiques détectés par Spectométrie X à Sélection d'Énergie et l'allergénicité des pollens. - Ann. Sci. Nat. Bot., Paris *13* ser. *9*, 133-152.

CERCEAU-LARRIVAL, M.-T., NILSSON, S., CAUNEAU-PIGOT, A., BERGGREN, B., DEROUET, L., VERHILLE, A.-M. and CARBONNIER-JARREAU, M.-C. (1991): The influence of the environment (natural and experimental) on the composition of the exine of allergenic pollen with respect to the deposition of pollutant mineral particles. - Grana *30*, 532-546.

GRATER, W.C. and STEMEN, T.R. (1967): The plant, the pollen and the patient. - Rev. Palaeobot., Palynol. *4*, 187-192.

JÁRAI-KOMLÓDI, M. (1991): First results of a study on airborne sporomorphs in Budapest, Hungary. - Grana *30*, 464-466.

JÁRAI-KOMLÓDI, M. and JUHÁSZ, M. (1993): *Ambrosia elatior* (L.) in Hungary (1989-1990). - Aerobiologia *9*, 75-78.

JÁRAI-KOMLÓDI, M. and MEDZIHRADSZKY, ZS. (1993): Aeropollen research in the air of Budapest in 1992. - Studia Botanica Hungarica *24*, 45-49.

KEDVES, M., BORBOLA, A., TRIPATHI, S.K.M. and MADHAV KUMAR (2000): Transmission electron microscopic studies on partially degraded pollen grains of *Phoenix sylvestris* LINN. - Plant Cell Biology and Development (Szeged) *12*, 93-103.

KEDVES, M. and PÁRDUTZ, Á. (2000): Chloroplast thylakoid membranes in the intine of ragweed pollen grains as a possible constituent in the biosynthesis of its extreme allergenic antigens. - Plant Cell Biology and Development (Szeged) *12*, 87-92.

KEDVES, M., PÁRDUTZ, Á. and MADARÁSZ, M. (2000): New data on the molecular symmetry and organization of the quasi-crystalloid skeleton of the sporoderm. - Plant Cell Biology and Development (Szeged) *11*, 184-192.

KNOX, R.B. and HESLOP-HARRISON, J. (1970): Pollen-wall proteins: localization and enzymatic activity. - J. Cell Sci. *6*, 1-27.

KNOX. R.B. and HESLOP-HARRISON, J. (1971): Pollen-wall proteins: localization of antigenic and allergenic proteins in the pollen grains walls of *Ambrosia* spp. (ragweeds). - Cytobios *4*, 49-54.

KNOX, R.B., HESLOP-HARRISON, J and REED, C. (1970): Localization of antigens associated with the pollen grain wall immunofluorescence. - Nature *225*, 1066-1088.

KNOX, R.B.WILLING, R.B. and ASHFORD, A.E. (1972): Role of pollen-wall proteins as recognition substance in interspecific incompatibility in poplars. - Nature *237*, 381-383.

LEUSCHNER, R.M. (1985): *L'Ambrosia* en Suisse. - Allergie et Immunologie *17*, 431-433.

LEUSCHNER, R.M., BOEHM, G. und MARI, R. (1990): Luftpollenbeobachtungen als Hinweis auf ein mögliches Vordringen von *Ambrosia* (=Ragweed) im Gebiet des Genfersees. - 2. Europäisches Pollenflug-Symposium 17/18. März 1989 Bad Honnef, 84-87.

MEZEI, GY., KOMLÓDI, M., BODOR, G. and CSERHÁTI, E. (1991): Correlation between concentrations of aeropollens and symptoms of allergenic rhinitis patients during ragweed season. - Medicina Thoracalis *44*, 471-476 (Hungarian with English summar).

MEZEI, GY., KOMLÓDI, M., PAPP, E. and CSERHÁTI, E. (1992). Late summer pollen and allergen spectrum in children with allergenic rhinitis and asthma in Budapest. - Pädiatrie u. Pädologie *27*, 75.

MOLNÁR, T. (1999): A levegőben található pollenek morfológiai vizsgálata és dinamikája. - Ph.D. Diss., Debrecen.

NILSSON, S., PRAGLOWSKI, J. and NILSSON, L. (1977): Atlas of Airborne Pollen Grains and Spores in Northern Europe. - Natur och Kultur, Stockholm.

O'ROURKE, M.K. (1996): New Frontiers in Palynology 23F - Medical Palynology. In: Palynology, principles and applicaions, eds.: JANSONIUS, J. and MCGREGOR, D.C. - A.A.S.P. Foundation Publ. Press, Salt Lake City, Utah *3*, 945-955.

PAYNE, W.W. and SKVARLA, J.J. (1970): Electron microscope study of *Ambrosia* pollen. - Grana *10*, 89-100.

PEHLIVAN, S. (1995): Türkiye'nin alerjen polenleri atlasi. - Matbaacilik Sanayi ve Ticaret Ltd. Sirketi, Ankara.

8. ESTUDIOS EXPERIMENTALES SOBRE GRANOS DE POLEN DE AMBROSIA CUMANENSIS H.B.K. DE EL SALVADOR

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Resumen

Se investigaron granos de polen de *Ambrosia cumanensis*, secos, hidratados y parcialmente degradados, de acuerdo con el método LM; además, se empleó el método TEM, para investigar un material de polen, parcialmente degradado. Basados en los estudios con LM, se estableció que la esporopolenina de la ectexina es menos resistente en contraste con la de *Ambrosia artemisiifolia*; pero desde el punto de vista taxonómico, no se observaron alternaciones en la morfología general del material experimental estudiado con el método LM. El estudio con el método TEM, de los granos parcialmente degradados con 2-aminoetanol, reveló la fina estructura de los granos de polen. En las cámaras de aire de la exina del polen se observaron microorganismos. En la intina existen unidades electrónicas globulares densas de origen desconocido.

Palabras claves: Palinología Experimental, reciente, *Ambrosia cumanensis*, LM y TEM.

Introducción

Existe un gran número de granos de polen del género *Ambrosia* que presentan un grado extremo alergénico. En 1967 GRATER y STEMEN señalaron que entre los granos de polen alergénicos, el del ragweed (*Ambrosia*) ha sido el más investigado intensamente. La ubicación de las proteínas antigénicas y alergénicas en la intina fue establecida por KNOX, HESLOP-HARRISON (1970, 1971), KNOX, HESLOP-HARRISON y REED (1970).

LAGOS (1975) publicó el dibujo del grano de polen de *Ambrosia cumanensis* y, con relación a esta especie, enfatizó lo siguiente, p. 37: "La altamisa constituye una planta muy importante en el campo de las alergias". O'ROURKE (1996) indicó que el género *Ambrosia*, con 45 especies, es el aeroalergeno más importante en Norteamérica. Se ha discutido, en primer lugar, que dos ácido-proteínas constituyen los principales antígenos (E y K). Durante nuestros estudios, empleando el método TEM en granos de polen, parcialmente disueltos, de *Ambrosia artemisiifolia* L. se observaron cloroplastos en la intina (KEDVES y PÁRDUTZ, 2000). El complejo molecular observado en la superficie de las membranas tilakoides, pueden constituir los factores, directos o indirectos, del extremo efecto alergénico de los granos de polen de *Ambrosia* (ragweed).

Durante las operaciones de simetría, el pentágono regular de la unidad biopolímera de la ectexina, parcialmente degradada del polen del ragweed, se rotó un pentágono

exunidad biopolímera globular (KEDVES, PÁRDUTZ y MADARÁSZ, 2000), lo cual fue un paso importante para los estudios del esqueleto metaestable cuasi-cristaloide de la ectexina.

El propósito de este estudio es el siguiente:

1. Establecer las alteraciones de la morfología de los granos de polen de *Ambrosia cumanensis*, después de diferentes tipos de experimentos.
2. Publicar los primeros datos, aplicando el TEM, de los granos de polen, parcialmente degradados, de *Ambrosia cumanensis*.
3. Comparar los resultados más recientes, con los anteriormente publicados.

Materiales y Métodos

El material del polen fue colectado por el Dr. J.A. LAGOS en San Salvador, El Salvador.

Los estudios con LM se realizaron como sigue:

Se investigó el diámetro de los granos de polen y la solubilidad de la esporopolenina de la exina de los siguientes granos de polen.

Diámetros de los granos de polen sin experimento:

Experimento N° 1/7-1373: granos de polen frescos montados en gel de glicerina hidratada al 39.6%. La temperatura para experimentos adicionales fue de 30° grados centígrados.

Experimento N° 1/7-1374: 5mg de granos de polen fueron hidratados con 5 ml de agua destilada durante 24 horas. Se estudiaron granos de polen coloreados con azul de metileno y granos sin colorear.

Experimento N° 1/7-1375-1377: 5mg de granos de polen + 1ml de 2-aminoetanol fueron sometidos durante: 24 horas a 1375; 48 horas a 1376 y 72 horas a 1377.

Experimento N° 1/7-1378-1380: 5mg de granos de polen + 1 ml de 2-aminoetanol, como en los casos 1375-1377; pero, después de lavados, fueron oxidados con 10 ml de $KMnO_4$ al 0.01%, durante 24 horas.

Experimento N° 1/7-1381-1383: igual a los experimentos anteriores (1378-1380); pero, al final, se agregó 1 ml de mercaptoetanol durante 24 horas.

Fueron preparados granos de polen, parcialmente degradados, para las investigaciones con el microscopio electrónico de transmisión. La fijación fue con OsO_4 aq. dil. 1% embebido en araldita (Durcupan, Fluka). Los cortes ultrafinos fueron hechos con un ultramicrótomo Porter Blum, con cuchillas de cristal, en el Laboratorio Em del Departamento de Biofísica del Centro de Investigaciones de la Academia Húngara de Ciencias. Las fotos fueron tomadas con un Tesla BS-540 (resolución 6-7 Å) y no se retocaron. En este trabajo presentamos los resultados del experimento N°: 1375.

En lo referente a la nomenclatura de la ultraestructura de la exina, se tomaron como base las publicaciones de: SKVARLA y LARSON (1965), SKVARLA y TURNER (1966), PAYNE y SKVARLA (1970), VASANTHY (1975), ROWLEY, DAHL y ROWLEY (1981) y ROWLEY, CLAUGHER y SKVARLA (1999).

Resultados

Resultados con LM.

La morfología básica de los granos de polen no ha alterado el punto de vista taxonómico (Láminas 8.1, fig. 1-16). Las alteraciones del diámetro de los granos de polen se resumen como sigue:

Diámetros	12.5	15.0	17.5	20.0	22.5	25.0	27.5	30.0 µm %
Granos de polen secos		2.5	21.5	49.0	23.5	3.5		
Experimento: 1/7-1373		4.0	50.5	40.75	4.75			
Experimento: 1/7-1374a			49.5	45.0	5.5			
Experimento: 1/7-1374b		6.0	46.0	38.5	9.5			
Experimento: 1/7-1375		29.5	47.0	23.5				
Experimento: 1/7-1376	0.5	4.5	27.0	32.0	21.5	8.0	5.0	1.5
Experimento: 1/7-1377		14.0	43.0	35.5	7.5			

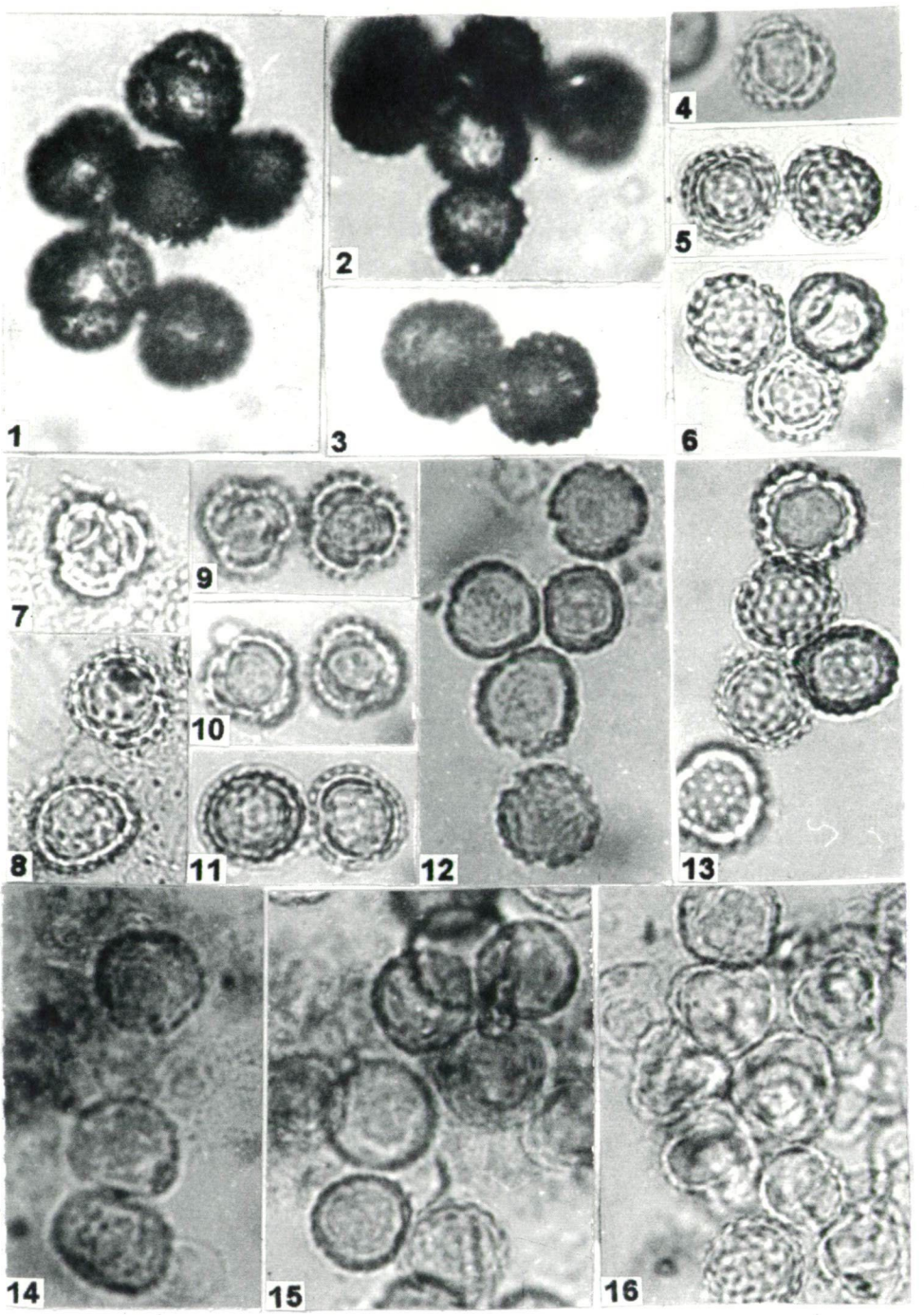
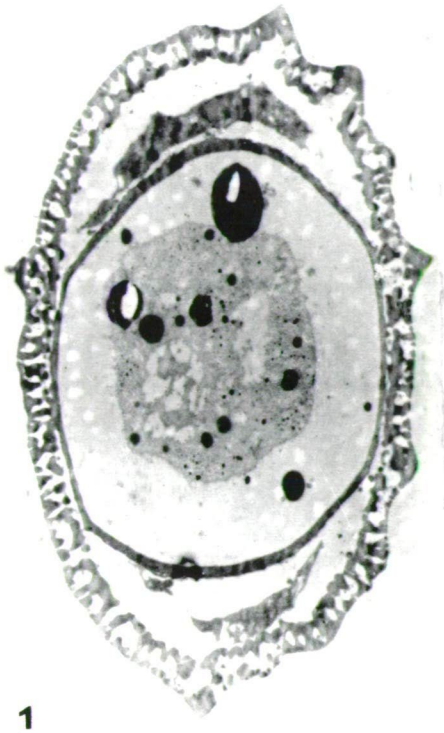


Lámina 8.1.



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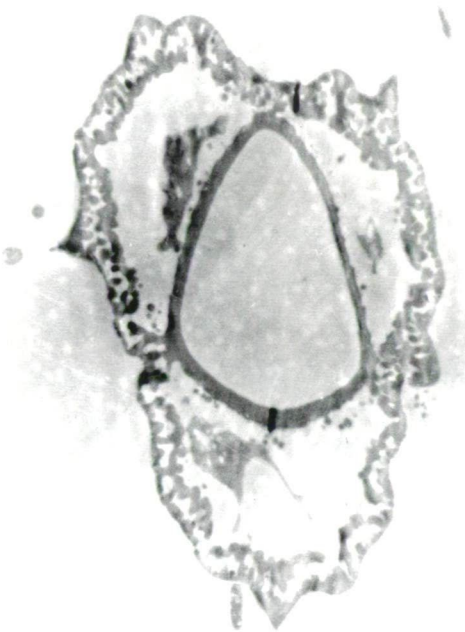

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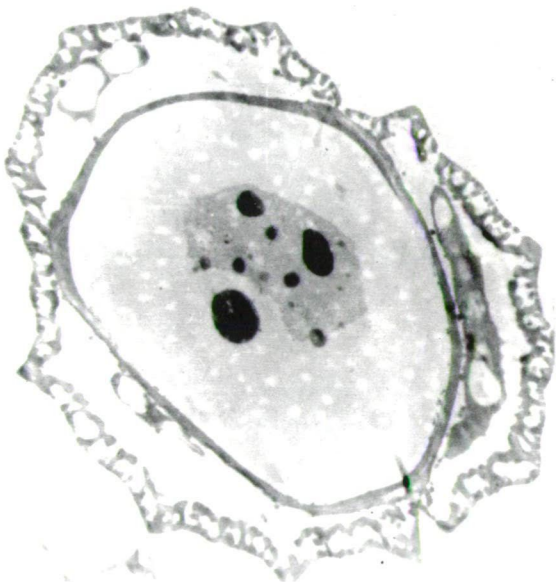

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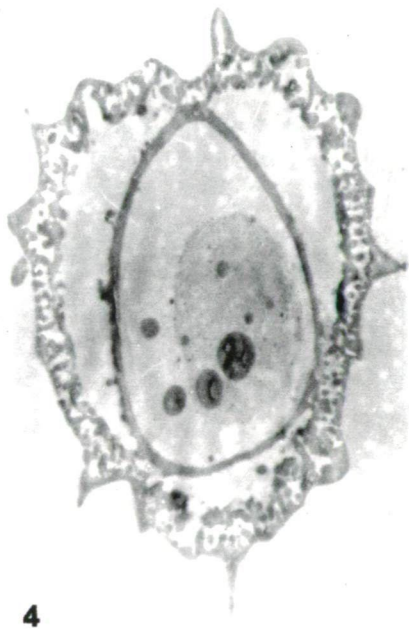

4

Lámina 8.2.

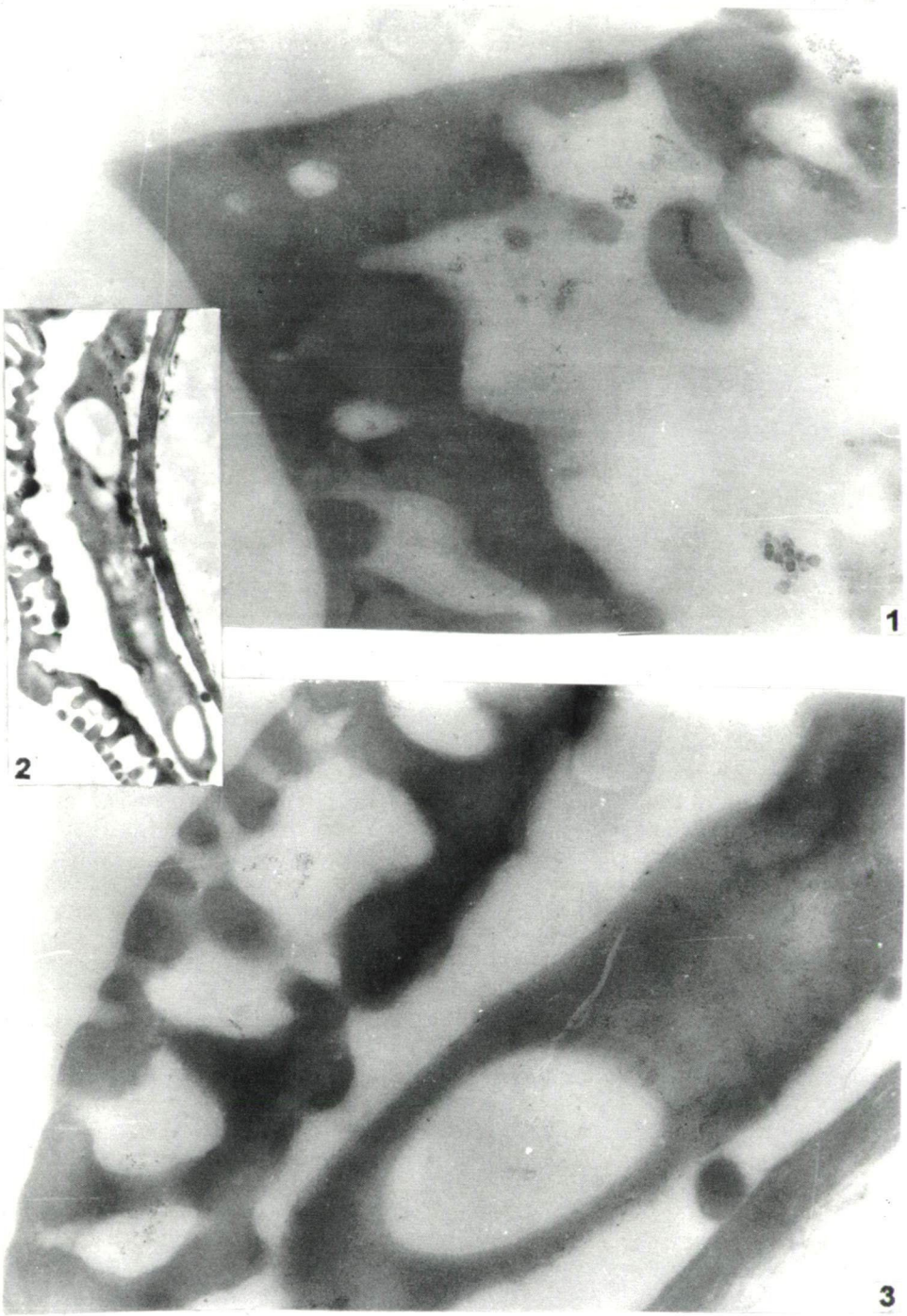

Lámina 8.3

Lámina 8.1.

Ambrosia cumanensis H.B.K., fotografías con microscopio óptico (LM), 1.000x.
1-3. Granos de polen secos.
4. Experimento N°: 1/7-1373a.
5, 6. Experimento N°: 1/7-1373b.
7-11. Experimento N°: 1/7-1374.
12, 13. Experimento N°: 1/7-1375.
14. Experimento N°: 1/7-1376.
15, 16. Experimento N°: 1/7-1377.

Lámina 8.2.

Ambrosia cumanensis H.B.K., fotografías con microscopio electrónico de transmisión (TEM) de granos de polen parcialmente degradados. Experimento N°: 1375.
1-4. Resumen general: fotografías de la ultraestructura de los granos de polen. 1. Negativo N°: 8333, 4500x, 2. Negativo N°: 8344, 4.500x, 3. Negativo N°: 8342, 4.500x, 4. Negativo N°: 8343, 4.500x.

Lámina 8.3.

Ambrosia cumanensis H.B.K. fotografías con microscopio electrónico de transmisión (TEM) de granos de polen partialmente degradados. Experimento N°: 1375.
1. Foto altamente aumentada de la escultura equinada del grano de polen. Negativo N°: 8337, 50.000x.
2. Foto que muestra el aspecto general de la ectexina del grano de polen, con microorganismos en la cámara del aire. Negativo N°: 8329, 85.000x.
3. Foto altamenete aumentada del área inter-apertural que muestra el téctum perforado, el estrato columelar infratectal y el estrato de la base. Se observa bien una parte de la ultraestructura del microorganismo, un gránulo denso electrónico, en la cámara de aire, y la intina.

Resultados con TEM (Láminas 8.2. y 3.)

El examen general de las fotografías de los granos de polen (Lámina 8.2., fig. 1-4) , muestra: la ectexina tectada, porforada y con espinas; el estrato de columelas infratectales; las cámaras de aire y el estrato de la base (Lámina 8.2., fig. 1-4). Los gránulos densos electrónicos no son frecuentes en el estrato infratectal (Lámina 8.2., fig. 2,3). La intina está hinchada. Algunas veces se observan gránulos densos electrónicos, de diferentes tamaños, en este estrato. Se han observado en el núcleo y en el protoplasma unidades de gránulos densos electrónicos (Lámina 8.2., fig. 1). Las fotografías con gran aumento muestran, muy bien, el tectum perforado, el estrato columelar infratectal y el estrato de la base (Lámina 8.3., fig. 3). Los microorganismos en las cámaras de aire se observan bien en la lámina 8.3., fig. 2-3. La fina estructura espinosa se distingue bien en la lámina 8.3., fig. 1.

Discusión y Conclusiones

1. La esporopolenina de la ectexina de *Ambrosia cumanensis*, es menos resistente comparada con la de *Ambrosia artemisiifolia* L. (KEDVES, PÁRDUTZ y HORVÁTH en proceso de publicación). Esto puede ser consecuencia de factores taxonómicos, ecológicos y ontogenéticos. La alteración molecular de la esporopolenina fue establecida hace mucho tiempo.

2. Vale la pena mencionar la estabilidad morfológica de nuestro material investigado con el microscopio óptico (LM).

3. Al comparar los presentes datos obtenidos con el microscopio electrónico de transmisión (TEM), con el mismo experimento de *Ambrosia artemisiifolia*, podemos señalar las similitudes en la estratificación de la ectexina, pero los gránulos densos electrónicos, no son tan frecuentes como en el estrato infratectal de *A. artemisiifolia*.

4. El microorganismo observado en la cámara de aire no es muy frecuente, pero interesante, y, probablemente, puede contribuir a la acción alergénica de este grano de polen.

Reconocimiento

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Referencias

GRATER, W.C. and STEMEN, T.R. (1967): The plant, the pollen and the patient. - Rev. Palaeobot. Palynol. *4*, 187-192.

KEDVES, M. and PÁRDUTZ, Á. (2000): Chloroplast thylakoid membranes in the intine of ragweed pollen grains as a possible constituent in the biosynthesis of its extreme allergenic antigens. - Plant Cell Biology and Development (Szeged) *12*, 87-92.

KEDVES, M., PÁRDUTZ, Á. and HORVÁTH, A. (2002): Transmission electron microscopy of partially degraded pollen grains of *Ambrosia artemisiifolia L.* - Plant Cell Biology and Development (Szeged), *14*, 49-58.

KEDVES, M., PÁRDUTZ, Á. and MADARÁSZ, M. (2000): New data on the molecular symmetry and organization of the quasi-crystalloid skeleton of the sporoderm. - Plant Cell Biology and Development (Szeged) *11*, 184-192.

KNOX, R.B. and HESLOP-HARRISON, J. (1970): Pollen-wall proteins: localization and enzimatic activity. - J. Cell. Sci. *6*, 1-17.

KNOX, R.B. and HESLOP-HARRISON, J. (1971): Pollen-wall proteins localisation of antigenic and allergenic proteins in the pollen grain walls of *Ambrosia* ssp. (ragweeds). - Cytobios *4*, 49-54.

KNOX, R.B., HESLOP-HARRISON, J. and REED, C. (1970): Localisation of antigens associated with the pollen wall immunofluorescence. - Nature *225*, 1066-1068.

LAGOS, J.A. (1975): Pólenes Anemófilos de El Salvador. - Ed. Universitaria, San Salvador, El Salvador.

O'ROURKE, M.K. (1996): New Frontiers in Palynology 23F - Medical Palynology. In: Palynology, principles and applications, eds.: JANSONIUS J. and MCGREGOR, D.C. - A.A.S.P. Foundation Publ. Press, Salt Lake City, Utah *3*, 945-955.

PAYNE, W.W. and SKVARLA, J.J. (1970): Electron microscope study of *Ambrosia* pollen. - Grana *10*, 89-100.

SKVARLA, J.J. and LARSON, D.A. (1965): An electron microscopic study of pollen morphology in the Compositae with special reference to the Ambrosiae. - Grana Palynol. *6*, 210-269.

SKVARLA, J.J. and TURNER, B.L. (1966): Systematic implicartions from electron microscopic studies ot Compositae pollen - A review. Ann. Mo. Bot. Gard. *53*, 220-244.

ROWLEY, J.R., CLAUGHER, D. and SKVARLA, J.J. (1999): Structure of the Exine in *Artemisia vulgaris* (Asteraceae): A Review. - Taiwania *44*, 1-21.

ROWLEY, J.R., DAHL, A.O. and ROWLEY, J.S. (1981): Substructure in exines of *Artemisia vulgaris* (Asteraceae). - Rev. Paleobot. Palynol. *35*, 1-38.

VASANTHY, G. (1975): Structure et nomenclature de la paroi sporopollinique: Asteraceae. - APLF Documents pour la réunion de Groupe de travail Morphologie sporopollinique et numériclature, Paris, 6-7 Octobre 1975, 44-46.

9. EXPERIMENTAL STUDIES ON THE MONOCOTYLEDONOUS MONOSULCATE POLLEN GRAINS

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Abstract

Pollen grains of *Lilium candidum* L. were the subject of our investigations. Fresh and heated pollen grains at 200 °C during 10 min., 1 hour, 5, 10, 25, 50 and 100 hours were investigated with the light microscope. Alterations in the polar axis and the P/E ratio were investigated. In general the pollen grains of this species are resistant, no taxonomically important alterations were observed as a consequence of the high temperature effect.

Key words: Palynology, recent, *Lilium candidum*, high temperature effect.

Introduction

The monosulcate form of the pollen grains is very important from the point of view of the angiosperm evolution. In general it may be taken as an early form. The peculiar reticulate type of the *Lilium* pollen grain is also a primitive caracteristic feature. Following BRENNER (1963) the fossil form-genus *Liliacidites* is represented in the Barremian-Aptian with two species (*peroreticulatus* and *reticulatus*). LAING (1976) established, that the monosulcate forms with clavate and/or reticulate sexines may be the first definitive angiosperm pollen. The pollen grains of the recent taxa of the Liliaceae were the subject of several different kinds of investigations. The basic LM morphological papers, among others, supported the role of the pollen morphology in the solution of taxonomic problems. For example: SHARMA (1967/68), SCHULZE (1975a,b, 1978a,b, 1980a,b), CHANDA and GHOSH (1976), TAKAHASHI (1987a,b), TAKAHASHI and SOHMA (1982, 1983), DIAZ LIFANTE, DIEZ and FERNÁNDEZ (1990) and KOSENKO (1999). Using the TEM method MEYER and YAROSHEVSKAYA (1976) established that the lamellar nexine in Liliaceae and Cyperaceae suggest a common ancestry. Following DICKINSON (1970), in *Lilium* the primexine is radially lamellate in probacules and vesiculate in the development of the muri. Further data concerning the development of the Liliaceae pollen grains was given by TAKAHASHI (1987), and SOHMA and TAKAHASHI (1982). HESLOP-HARRISON (1973) established radical chages in *Lilium* cytoplasmatic membranes of the meiocyte with a prophase decline in ribosome number. Following KNOX (1973), the thin intines of Liliaceae and Amaryllidaceae show less enzymatic activity, although in the large grains of *Lilium* spp. the hydrobases are readily detectable in the colpal zone. The protoplast of *Lilium longiflorum* THUNB. and in vitro regenerations of the cellulozic walls were investigated by MIKI-HIROSIGE, NAKAMURA and TANAKA

(1988). There are several papers on the orbicules of the pollen grains of *Lilium* (CLÉMENT and AUDRAN, 1992, 1993a,b,c).

The secretion from the pistil of *Lilium longiflorum* was published by MIKI-HIROSIGE, HOEK and NAKAMURA (1987). MIKI-HIROSIGE (1961) investigated the pollen germination and pollen tube growth in pistil, stigma, style and ovary slices. ROSEN (1973a), p. 177, established the following: "*Lilium longiflorum* pollen cytoplasm possesses numerous lipid droplets but lack starch". The metabolism of germinating *Lilium* pollen was described by DAVID (1973). DASHEK, HARWOOD, and ROSEN (1973) pointed out the significance of the wall-bound hydroxyproline-containing glycoproteide in *Lilium* pollen tube elongation.

The first data on the chemistry of the pollen wall was published by JOHN (1814) from a Liliaceae pollen grain (*Tulipa*). *Lilium henryi* THUNB. was the subject of the study of BROOKS and SHAW (1968, 1973, 1978). It was established, that the precursors of the sporopollenin are β-caroten and its esters. The precursor importance of the phenylalanine in the pollen grains of the genus *Tulipa* was demonstrated by RITTSCHER, GUBATZ and WIERMANN (1987). SOUTHWORTH (1985, 1986) acetolyzed exines of *Lilium longiflorum* THUNB. partially extracted with hot 2-aminoethanol. The residual material was investigated with the TEM method.The lattice-like substructure of interconnected granules was composed of different kinds of polygons. Previously we investigated the high temperature effect on monosulcate, tricolpate and tricolporate pollen grains (KEDVES et al., 1993) and it was established, that after heating the monosulcate angiosperm pollen grains, such as *Magnolia, Allium* and *Chamaedorea*, are similar to early Mesozoic gymnosperm pollen grains.

The aim of this paper is to show new results on the secondary alterations of the monosulcate monocotyledonous pollen grains.

Materials and Methods

The material investigated was collected by Miss. A. HEGEDÜS, and I. OLÁH in the Botanical Garden of the University of Szeged, on the 9 June 1990. The pollen material was frozen at -20 °C. The experiments were started on 21 January 1992. Fresh (1285) and heated pollen grains were the subject of our investigations. Temperature: 200 °C, Length of time: 10 minutes (1286), 1 hour (1287), 5 hours (1288), 10 hours (1289), 25 hours (1290) and 50 hours (1291), 100 hours (1292). The distribution in percentages of the P/E ratio and the variation of the polar axis were measured.

Results

Pollen grains, as well established during previous investigations, are monosulcate and in surface sculpture, characteristically reticulate (Plate 9.1., figs. 1-3). The mesh of the reticula in the inter-apertural area (proximal pole) is about 3.6-8.4 μm (Plate 9.1., fig. 3), in the apertural area it is smaller 1.6-2.8 μm (Plate 9.1., figs. 1,2). The amb of the fresh pollen grains is spindle shaped (Plate 9.2., fig. 1). The polar axis varies from 62.5 μm to 100.0 μm (Table 9.1.), the P/E ratio is 1.1-2.0, maximum, 28.0% at 1.3. (Table 9.2).

Pollen grains heated for 10 minutes (Plate 9.2., fig. 3, table 9.1., 9.2)

The length of the polar axis increased from 70.0 μm to 122.5 μm, 11.5% at 95.0.μm, 10.5% at 105.0 μm. Important changes happened to the P/E ratio, this value is 2.0, in the greatest part of the pollen grains (15.5%).

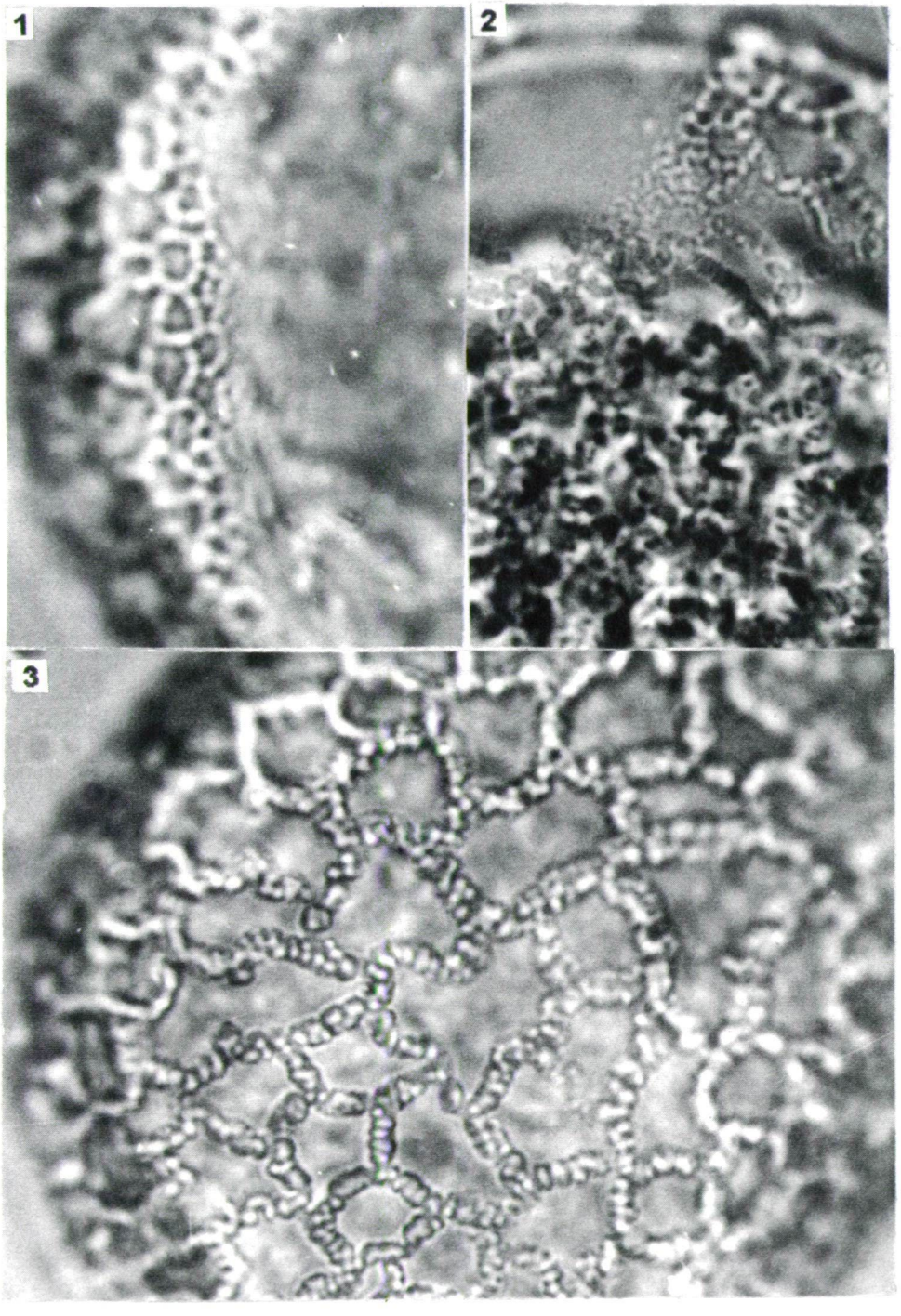

Plate 9.1.

Plate 9.2.

Plate 9.1.

1-3. *Lilium candidum* L., fresh pollen grains, 2500x.
1,2. Detail from the apertural area. The reduced diameter of the mesh of the reticula is well illustrated.
3. Surface ornamentation of the pollen grain on the proximal surface.

Plate 9.2.

1-12. *Lilium candidum* L. 750x.
1,2. Fresh pollen grains.
3. Pollen grain heated for 10 minutes.
4. Pollen grain heated for 1 hour.
5. Pollen grain heated for 5 hours.
6. Pollen grain heated for 10 hours.
7,8. Pollen grains heated for 25 hours.
9,10. Pollen grains heated for 50 hours.
11,12. Pollen grains heated for 100 hours.

Pollen grains heated for 1 hour (Plate 9.2., fig. 4, table 9.1., 9.2)

The length of the polar axis is nearly the same as in the previous experiment, from 67.5 µm to 120.0 µm, maximum (11.5%) is at 102.5 µm. The P/E ratio varies from 1.2 to 2.6, maximum (13.5%) at 2.2.

Pollen grains heated for 5 hours (Plate 9.2., fig. 5, table 9.1., 9.2)

The length of the polar axis varies from 70.0 µm, to 115.0 µm, the maximum is a little decreased, 15.0% at 100.0 µm. The P/E ratio is also reduced in contrast to the previous experiment, 18.5% is at 1.9.

Pollen grains heated for 10 hours (Plate 9.2, fig. 6, table 9.1, 9.2)

The length of the polar axis is 70.0 µm to 120.0 µm,the maximum is 87.5 µm (12.0%) therefore the previously mentioned trend continuted. The maximal value of the P/E ratio (1.8) is 14.5%.

Pollen grains heated for 25 hours (Plate 9.2., figs. 7,8, table 9.1, 9.2)

The length of the polar axis is 70.0 µm to 117.5 µm, the maximum (12.0%) is at 100 µm. This is a quite similar to the previous experiment. P/E ratio from 1.1 - 2.2, maximum (14.5%) at 1.9.

Pollen grains heated for 50 hours (Plate 9.2., figs. 9,10, table 9.1., 9.2)

Polar axis from 60.0 µm to 112.5 µm, maximum (11.0%) at 95.0 µm. High percentage (105%) was measured at 82.5 µm, a trend of reduction in consequence of the heating may be established.The P/E ratio maximum is identical with the previous experiment, 17.5% at 1.9.

Pollen grains heated for 100 hours (Plate 9.2., figs. 11,12, table 9.1., 9.2)

A remarkable diminution was observed in the length of the polar axis. Percentages over 10% were observed at 80.0 - 87.5 µm, altogether 47.5%.The P/E ratio is nearly the same as previously. This character has not changed significantly from the heating for 5 hours.

	60.0	62.5	65.0	67.5	70.0	72.5	75.0	77.5	80.0	82.5	85.0	87.5	90.0	92.5	95.0	97.5	100.0	102.5	105.0	107.5	110.0	112.5	115.0	117.5	120.0	122.5
1285		0.5	0.5	1.0	5.0	5.5	12.0	17.0	18.5	13.0	12.0	9.5	2.5	1.5	0.5		1.0									
1286					0.5	1.5	1.5	2.0	1.5	3.0	5.0	6.5	7.5	7.5	11.5	6.5	8.0	9.0	10.5	6.0	5.0	3.0	2.5		0.5	1.0
1287				0.5	0.5	0.5	0.5	2.0	2.5	4.0	3.0	8.0	6.0	3.0	9.0	8.0	8.5	11.5	8.0	6.5	5.0	10.0		2.0	1.0	
1288					0.5	1.5	1.5	3.5	3.5	3.0	4.0	7.5	9.0	9.0	10.0	6.5	15.0	4.0	6.5	7.0	5.0	2.0	1.0			
1289					0.5	0.5	4.5	3.5	3.0	6.0	4.0	12.0	5.5	7.0	8.0	7.5	11.5	6.5	7.5	5.5	1.0	1.0	4.0	0.5	0.5	
1290					1.0	1.5	4.5	5.0	2.0	4.0	6.5	9.5	5.5	7.5	11.5	7.0	12.0	3.5	6.5	6.0	2.0	3.0	1.0	0.5		
1291	0.5	0.5	1.0	2.5	4.0	2.5	6.5	3.0	7.0	10.5	12.5	13.5	6.0	6.5	11.0	2.0	2.5	3.5	1.0	2.5		1.0				
1292	0.5	0.5	1.0	2.5	7.0	4.0	9.0	5.0	11.5	11.5	14.0	11.5	8.0	5.0	3.0	4.5	1.0			0.5						

Table 9.1.

The percentages of the length of the polar axis of the pollen grains. Explanation: 1285, fresh pollen grain, 1286, pollen grains heated for 10 minutes, 1287, pollen grains heated for 1 hour, 1288, pollen grains heated for 5 hours, 1289, pollen grains heated for 10 hours, 1290, pollen grains heated for 25 hours, 1291, pollen grains heated for 50 hours, 1292, pollen grains heated for 100 hours.

P/E ratio	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9
Experiment No																			
1285	12.0	23.5	28.0	19.0	9.0	5.0	1.5	1.0	0.5	0.5									
1286	1.0	2.0	1.5	2.5	7.0	6.5	11.5	13.0	12.5	15.5	11.5	6.5	4.0	2.5	2.0		0.5		
1287		2.5	1.0	1.5	3.0	3.0	11.5	13.0	7.5	21.0	10.5	13.5	4.5	4.0	2.0	1.5			
1288		0.5	4.0	4.0	1.5	7.0	7.0	9.0	18.5	15.0	12.5	13.5	4.0	1.5	1.5	0.5			
1289	0.5	1.5	2.5	5.0	8.0	3.5	9.0	14.5	13.5	10.0	12.5	10.0	6.0	2.0	1.0				0.5
1290	0.5	6.0	6.5	4.0	7.0	6.5	11.5	11.5	14.5	11.0	10.0	7.0	4.0						
1291		3.0	1.5	4.0	5.5	9.0	7.5	9.5	17.5	15.0	11.5	8.0	5.0	2.5	0.5				
1292	0.5	1.0	3.0	4.5	2.5	9.5	11.0	12.5	13.0	11.0	12.0	6.0	8.5	2.5	2.0	0.5			

Table 9.2.

The percentages of the P/E ratio of the pollen grains. Explanation 1285, fresh pollen grains. 1286, pollen grains heated for 10 minutes, 1287, pollen grains heated for 1 hour, 1288, pollen grains heated for 5 hours, 1289, pollen grains heated for 10 hours, 1290, pollen grains heated for 25 hours, 1291, pollen grains heated for 50 hours, 1292, pollen grains heated for 100 hours.

Discussion and Conclusions

1. In the introduction, an attempt was made to review the most important fields of investigation concerning monosulcate pollen grains, in particular the Liliaceae. The phylogenetic importance of this kind of pollen grains is remarkable, cf. M. VAN CAMPO (1967, 1976).

2. A long time ago KIRCHHEIMER, 1933 that during the fossilization process the high temperature may provoke secondary alterations. In order to interpret the palynological data from metamorphic rocks so called model experiments are necessary.

3. The pollen grains of *Lilium* seem to be morphologically resistant. Important changes in the quantitative morphological characteristic features happen after 10 minutes of heating. The polar axis and the P/E ratio of fresh and the briefly heated pollen grains are extremely different. The averages of the polar axis and the P/E ratio are summarized as follows.

Duration of heating	Polar axis	P/E ratio
0	80.125	1.3225
10minutes	96.95	1.875
1hour	98.325	1.955
5hours	95.125	1.92
10hours	94.35	1.88
25hours	93.275	1.78
50hours	86.225	1.873
100hours	82.3	1.89

Based on these data no important changes may be observed in the quantitative data of the heated pollen grains, in contrast to the evaluation of the detailed data, see Table 9.1., and 9.2.

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References

BRENNER, G.J. (1963): The spores and pollen of the Potomac Group of Maryland. - Maryland Board Nat. Resources, Dept. Geol. Mines Water Resources Bull. *27*, 1-215.

BROOKS, J. and SHAW, G. (1968): The post-tetrad ontogeny of the pollen wall and the chemical structure of the sporoderm of *Lilium henryi*. - Grana Palynologica *8*, 227-234.

BROOKS, J. and SHAW, G. (1973): The role of sporopollenin in Palynology. - Problems of Palynology, 80-91.

BROOKS, J. and SHAW, G. (1978): Sporopollenin: a review of its chemistry, palaeochemistry and geochemistry. - Grana *17*, 91-97.

CHANDA, S. and GHOSH, K. (1976): Pollen morphology and its evolutionary significance in Xanthorrhoeaceae. In: The evolutionary significance of the exine, eds.: FERGUSON, I. K. and MULLER, J. - Linnean Soc Ser. *1*, 527-560.

CLÉMENT, C. et AUDRAN, J.C. (1992): Apports de la cytochimie à la connaissance des orbicules dans l'anthère de *Lilium* (Liliacées). 1. Le coeur orbiculaire. - Bull. Soc. bot. Fr., *139*, Lettres bot. (415), 369-376.

CLÉMENT, C. and AUDRAN, J.C. (1993a): Electron microscope evidence for a membrane around the core of the Ubisch body in *Lilium* (Liliaceae). - Grana *32*, 311-314.

CLÉMENT, C. and AUDRAN, J.C. (1993b): Cytochemical and ultrastructural evolution of orbicules in *Lilium* - Pl. Syst. Evol. [Suppl.] *7*, 63-74.

CLÉMENT, C. and AUDRAN, J.C. (1993c): Orbicule wall surface characteristics in *Lilium* (Liliaceae) An ultrastructural and cytochemical approach. - Grana *32*, 348-353.

DASHEK, W.V., HARWOOD, H.I. and ROSEN, W.G. (1973): The Significance of a Wall-bound, Hydroxyproline-containing Glycopeptide in Lily Pollen Tube Elongation. - In: Pollen: Development and Physiology, ed.: J. HESLOP-HARRISON. - London Butterworths, 194-200.

DAVID, B. (1973): Metabolism of Germinating Lily Pollen: Pollen Enzymes. In: Pollen Development and Physiology, ed.: J. HESLOP-HARRISON. - London, Butterworths, 190-193.

DIAZ LIFANTE, Z., DIEZ, M.J. y FERNÁNDEZ, I. (1990): Morfología polinica de las subfamilias Melanthoideae y Asphodeloideae (Liliaceae) en la Península Ibérica y su importancia taxonomica. - Lagascalia *16*, 211-225.

DICKINSON, H.G. (1970): Ultrastructural aspects of primexine formation in the microspore tetrad of *Lilium longiflorum*. - Cytobiologie *1*, 437-449.

HESLOP-HARRISON, J. (1973): The cytoplasm and its organelles during meiosis. In: Pollen: Development and Physiology, ed.: J. HESLOP-HARRISON. - London, Butterworths, 16-35.

JOHN, J.F. (1814): Über Befruchtensstaube nebst eine Analyse des Tulipen Pollens. - J. Chem. Physik *12*, 244-261.

KEDVES, M., BORBOLA, A., TRIPATHI, S.K.M. and MADHAV KUMAR (2000): Thermal effect on some extant palm pollen. - Plant Cell Biology and Development (Szeged) *11*, 166-183.

KEDVES, M. and KÁROSSY, Á. (1997): X-Ray effect on the LM morphology of some angiosperm pollen grains I. - Plant Cell Biology and Development (Szeged) *8*, 86-90.

KEDVES, M., TÓTH, A., MÉSZÁROS, K., BORBOLA, A. and AILER, P. (1993). Recent modelling of the major evolutionary degrees of early angiosperm pollen types. - Plant Cell Biology and Development (Szeged) *4*, 64-73.

KIRCHHEIMER, F (1933): Die Erhaltung der Sporen und Pollenkörner in den Kohlen sowie ihre Veränderungen durch die Aufbereitung. - Bot. Arch. *35*, 134-187.

KNOX, R.B. (1973): Pollen Wall Enzymes: taxonomic distribution and physical localisation. In: Pollen: Development and Physiology, ed.: J. HESLOP-HARRISON. - London, Butterworths, 171-173.

KOSENKO, V.N. (1999): Contributions to the pollen morphology and taxonomy of the Liliaceae. - Grana *38*, 20-30.

LAING, J.F. (1976): The stratigraphic settling of early angiosperm pollen. In: The evolutionary significance of the exine eds.: I.K. FERGUSON and J. MULLER. - Linnean Soc. Ser. *1*, 15-26.

MEYER, N.R. and YAROSHEVSKAYA, A.S. (1976): The phylogenetic significance of the development of pollen grain walls in Liliaceae, Juncaceae and Cyperaceae. In: The evolutionary significance of the exine eds.: I.K. FERGUSON and J. MULLER. - Linnean Soc. Ser. *1*, 91-100.

MIKI, H. (1954): A study of tropism of pollen tubes to the pistil I. Tropism in *Lilium*. - Bot. Mag. Tokyo *67*, 791-792.

MIKI, H. (1961): A study of tropism of pollen tubes to the pistils IV. Tropism in Different Species. - Mem. Coll. Sci. Univ. of Kyoto, B, *28*, 1 (Biology), 105-118.

MIKI-HIROSIGE, H. (1961): Pollen germination and pollen tube growth in the presence of pistil slices in vitro. - Mem. Coll. Sci. Univ. Kyoto B, *28*, 3, (Biology), 375-388.

MIKI-HIROSIGE, H. (1962): A study of tropism of pollen tubes to the pistils VI. Behavior of pollen grains to stigma of different stages in development. - Mem. Coll. Sci. Univ. Kyoto, Ser. B, *29*, 75-80.

MIKI-HIROSIGE, H., HOEK, I. H. S. and NAKAMURA, S. (1987): Secretions from the pistil of *Lilium longiflorum*. - Amer. J. Bot. *74*, 1709-1715.

MIKI-HIROSIGE H., NAKAMURA, S. and TANAKA, I. (1988): Ultrastructural research on cell wall regeneration by cultured pollen protoplast of *Lilium longiflorum*. - Sex Plant Reprod. (1988) *1*, 36-45.

RITTSCHER, M., GUBATZ, S. and WIERMANN, R. (1987): Phenylalanin a precursor of sporopollenin in *Tulipa* ca. Apeldoorn. - XIV. Internat. Bot. Congr., Berlin (West), Germany, abstr. 51.

ROSEN, W.G. (1973a): Pollen Tube Growth and Tissue Structure. - In: Pollen: Development and Physiology, ed.: J. HESLOP-HARRISON. - London, Butterworths, 177-185.

ROSEN, W.G. (1973b): Pistil-pollen interactions in *Lilium*. In: Pollen: Development and Physiology, ed.: J. HESLOP-HARRISON.. - London, Butterworths, 239-254.

ROWLEY, J.R. (1976): Dynamic changes in pollen wall morphology. In: The evolutionary significance of the exine, eds.: FERGUSON, I.K. and MULLER, J. -Linnean Soc. Ser. *1*, 39-66.

SCHULZE, W. (1975a): Beiträge zur Taxonomie der Liliifloren I. Asphodelaceae. - Wiss. Ztschr. Friedrich-Schiller Univ. Jena Math, Nat. R. *24*, 403-415.

SCHULZE, W. (1975b): Beiträge zur Taxonomie der Liliifloren II. Colchicaceae. - Wiss. Ztschr. Friedrich-Schiller Univ. Jena, Math. Nat. R; *24*, 417-428.

SCHULZE, W. (1978a): Beiträge zur Taxonomie der Liliifloren III. Alstroemeriaceae. - Wiss. Ztschr. Friedrich-Schiller Univ. Jena, Math. Nat. R; *27*, 79-85.

SCHULZE, W. (1978b): Beiträge zur Taxonomie der Liliifloren IV. Melanthiaceae. - Wiss. Ztschr. Friedrich-Schiller Univ. Jena Math. Nat. R; *27*, 87-95.

SCHULZE, W. 1980a): Beiträge zur Taxonomie der Liliifloren V. Alliaceae. - Wiss. Ztschr. Friedrich-Schiller Univ. Jena Math. Nat. R; *29*, 595-606.

SCHULZE, W. (1980b): Beiträge zur Taxonomie der Liliifloren VI. Der Umfang der Liliaceae. - Wiss. Ztschr. Friedrich-Schiller Univ. Jena Math. Nat. R; *29*, 607-636.

SHARMA, M. (1967/68): Pollen morphology of Indian Monocotyledons. - J. of Palynol. Spec. Vol.

SOHMA, K. and TAKAHASHI, M. (1982): Exine formation of *Smilacina yesoensis* FR. et SAV. and *S. Japonica* A. GRAY (Liliaceae). - Sci. Rep. Tohoku Univ. 4th ser. Biology, *38*, 165-172.

SOUTHWORTH, D. (1985): Pollen exine substructure. I. *Lilium longiflorum*. - Amer. J. Bot. *72*, 1274-1283.

SOUTHWORTH, D. (1986): Substructural organization of pollen exines. - Pollen and Spores Form and Function, 61-69.

TAKAHASHI, M. (1987): Pollen morphology in the genus *Erythronium* (Liliaceae) and its systematic implications. - Amer. J. Bot. *74*, 1254-1262.

TAKAHASHI, M. and SOHMA, K. (1982): Pollen morphology of the genus *Clintonia* (Liliaceae). - Sci. Rep. Tohoku Univ. 4th ser. Biology, *385*, 157-164.

TAKAHASHI, M. and SOHMA, K. (1983): Pollen morphology of the genus *Smilacina* (Liliaceae). - Sci. Rep. Tohoku Univ. 4th. ser. (Biology) *38*, 191-218.

VAN CAMPO, M. (1967): Pollen et classification. - Rev. Palaeobot. Palynol. *8*, 52-75.

VAN CAMPO, M. (1976): Patterns of pollen morphological variation within taxa. In: The evolutionary significance of the exine, eds.: FERGUSON, I.K. and MULLER, J. - Linnean Soc. Ser. *1*, 125-128.

10. VARIATIONS IN LM MORPHOLOGY OF PARTIALLY DEGRADED PALM POLLEN GRAINS FROM INDIA

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Abstract

Partially degraded pollen of *Arenga pinnata, Borassus flabellifer* and *Caryota urens* were studied with an object to observe the morphological changes. Degradation was achieved in the three sets of experiments. In the first experiment pollen were treated with 2-aminoethanol for a period(s) of 1, 2 and 3 days. This treatment was followed by oxidation of pollen with the help of 1% dil. $KMnO_4$ for a period of 24 hours. This constituted the second experiment. In the third experiment pollen grains treated with 2-aminoethanol were kept in merkaptoethanol for 24 hours. Pollen were treated with 50% glycerine for 30 days to study the nature of exine, intine and protoplasmic contents. Based on the features observed after these experiments, four groups were identified in the studied pollen grains. These are: A - unchanged monosulcate pollen grains, B - open pollen grains with endexine and protoplasm contained within the pollen, C - open pollen with ectexine and without endexine and protoplasm and D - the endexine and protoplasm without the ectexine. Statistical data of pollen constituting each group has been represented in the Text-fig. 10.1. Alteration and variation in morphology of the studied pollen have been discussed.

Key words: Palynology, recent, Arecales, partial degradation, LM.

Introduction

Pollen exine is very non-reactive and resistant to most of the chemicals. The inert and non-destructible nature of the exine is responsible for the survival of the pollen grains in the geological history. Pollen corrosion or pollen destruction in nature has been related with determination of pollen wall composition (STANLEY and LINSKENS, 1974). Studies have identifed differential susceptibility to degradation in nature (ROWLEY and PRIJANTO, 1977). Oxidation of pollen walls with the help of different chemicals used in experimental studies has thus been correlated with resistance to natural corrosion.

In the present communication results of the experimental studies on extant pollen of *Arenga pinnata, Borassus flabellifer*, and *Caryota urens* have been summarised. These pollen belong to the family Arecaceae and were collected from India. During the investigations these pollen were partially degraded with the help of 2-aminoethanol, $KMnO_4$ and merkaptoethanol. The pollen were also treated with 50% glycerine. Details of the experiments are given in the later part of the text under materials and methods.

The aim of the conducted experiments was to observe changes in the pollen morphology and degradation affects after treating these with 2-aminoethanol (for duration of 24, 48 and 72 hours) followed by treatment with dil. $KMnO_4$ (1%) or merkaptoethanol. In most of the pollen as a sequel to these treatments the colpus was observed to be widely

open and in some pollen probably intine and protoplasm exuded out. As a result of hydration the swelling and extrusion of intine and protoplasm has been reported earlier also (DUHOUX, 1975; SOUTHWORTH, 1988; KEDVES et al., 1997; EL-GHAZALY et al., 1998; AMBWANI and KUMAR, 1991). This phenomenon was termed as "Duhoux effect" by KEDVES et al. (1997).

Materials and Methods

Materials for the investigations were collected by S.K.M. TRIPATHI and M. KUMAR from India. The experiments were carried out as follows:

Experiment numbers: *Borassus flabellifer* T-12-48 - T-12-58, *Arenga pinnata* T-12-59 - T-12-69, *Caryota urens* T-12-70 - T-12-80. Five milligrams dried pollen material were used for each experiment. The experiments were conducted at 30°C.

Fresh, non-experimental pollen grains: T-12-48, T-12-59, T-12-70.

Experiment numbers: T-12-49, T-12-60, T-12-71. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 24 h.

Experiment numbers: T-12-50, T-12-61, T-12-72. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 48h.

Experiment numbers: T-12-51, T-12-62, T-12-73. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 72h.

Experiment numbers: T-12-52, T-12-63, T-12-74. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 24h; washing, + 10 ml $KMnO_4$, 1%, length of time 24h.

Experiment numbers: T-12-53, T-12-64, T-12-75. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 48h; washing, + 10 ml $KMnO_4$, 1%, length of time 24h.

Experiment numbers: T-12-54, T-12-65, T-12-76. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 72h; washing, + 10 ml $KMnO_4$, 1%, length of time 24h.

Experiment numbers: T-12-55, T-12-66, T-12-77. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 24h; washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment numbers: T-12-56, T-12-67, T-12-78. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 48h; washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment numbers: T-12-57, T-12-68, T-12-79. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 72h; washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment numbers: T-12-58, T-12-69, T-12-80. - 5 mg pollen material + 5 ml glycerine, 50%, length of time 30 days.

Results

A. Non-experimental studies (Text - fig. 10.1.) - Normal pollen grains of *Arenga pinnata, Borassus flabellifer* and *Caryota urens* were studied after mounting these in hydrated glycerine-jelly (39.6%). Most of the *Arenga pinnata* pollen at this stage were found with open colpus, of which about half of them without the intine and protoplasm. However, no protoplasm contained within the intine was noticed indicating the possible degradation of intine and protoplasmic contents in at least 30% pollen. *Borassus flabellifer* pollen were observed in normal condition in comparatively higher number but in most of them colpus was open and pollen were without intine and protoplasm. Degradation of intine and protoplasm was observed in 40% pollen. *Caryota urens* pollen in glycerine-jelly mounts were mostly open and contained the intine and protoplasm in about half of the specimens. Degradation of intine and protoplasm in about half number of specimens is thus indicated.

B. Experimental studies

I. In the first experiment pollen grains of *Arenga pinnata* (Plate 10.1., figs. 1,2,6,11,14,17,18), *Borassus flabellifer* (Plate 10.2., figs. 1,2,4,9; Plate 10.3., figs. 1,2)

and *Caryota urens* (Plate 10.4., figs. 1-3, 8-11) were partially degraded with the help of 2-aminoethanol for a duration of 24, 48 and 72 hours. These pollen were studied under the light microscope to observe the morphological changes. After this experiment *Arenga pinnata* pollen were observed to possess the open colpus but about half of the specimens lost intine and protoplasm after the duration of 24 and 72 hours. Surprisingly after 48 hours treatment the results were a little different as the destruction of intine and protoplasm was not as after 24 and 72 hours treatment.

As a result of this experiment the pollen of *Borassus flabellifer* were most commonly observed to have open colpus and did not possess the intine and cytoplasm. Destruction of intine and cytoplasm was most effective after the duration of 72 hours.

Almost all pollen of *Caryota urens* had the open colpus but only half of these possessed intine and protoplasm. Degradation of intine and protoplasm was observed in equal number of pollen after 24 and 72 hours whereas, after 48 hours these contents were degraded in more pollen.

II. In this experiment pollen grains were treated with 2-aminoethanol for the periods of 24, 48 and 72 hours followed by oxidation with the help of 1% dil. $KMnO_4$ for a duration of 24 hours. After this experiment most of the pollen grains of *Arenga pinnata* (Plate 10.1., figs. 3, 4, 7-9, 13, 20, 21) exhibited degradation of intine and protoplasm. Colpus was open in almost all the pollen grains. Degradation of intine and protoplasm was almost complete after 72 hours. Similar kind of degradation trend was observed in the pollen of *Borassus flabellifer* (Plate 10.2., figs. 3, 10, Plate 10.3., figs. 3, 4, 9). In these pollen degradation was achieved in 24 hours only.

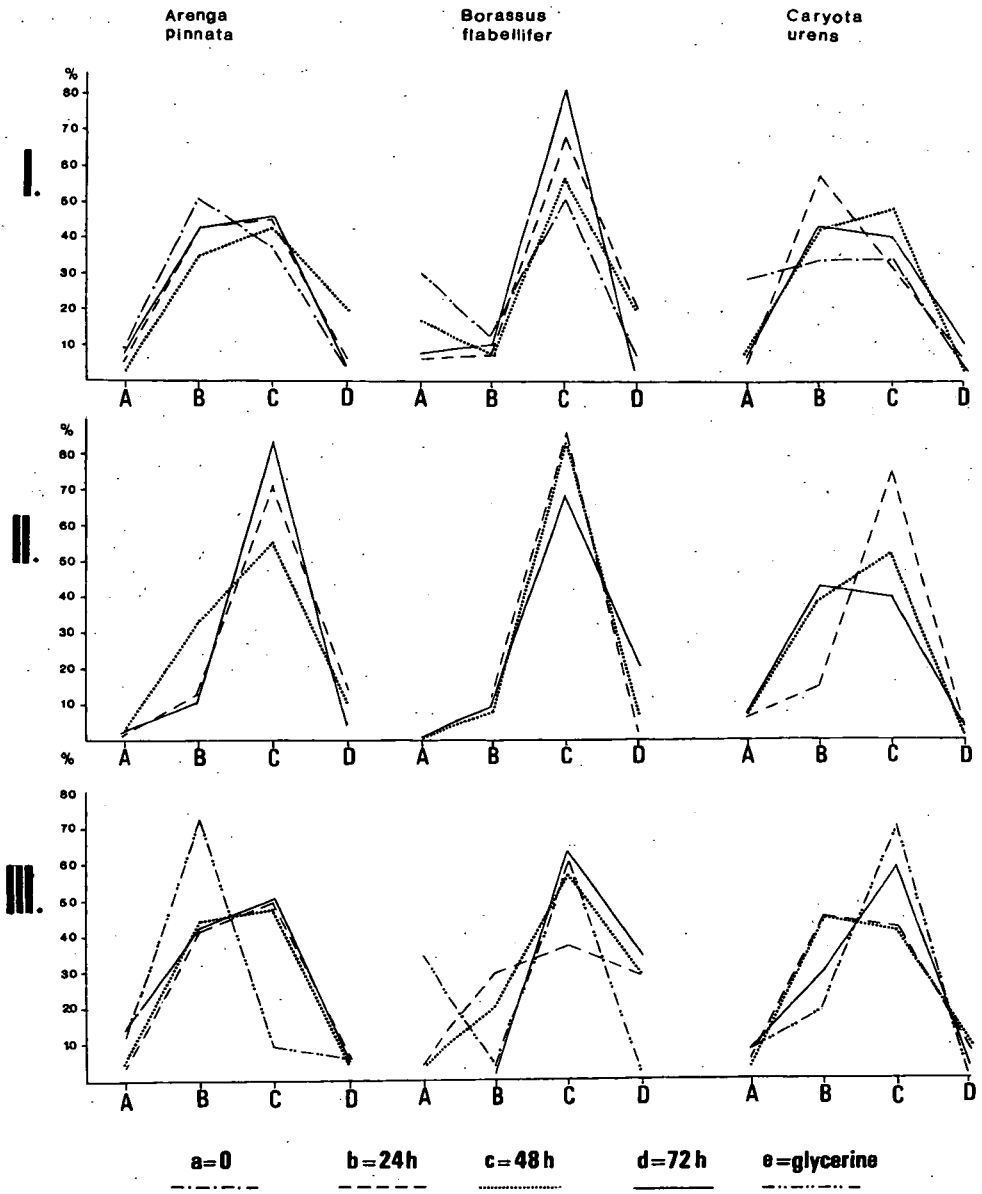
Pollen grains of *Caryota urens* (Plate 10.4., figs. 4-6, 12-21, 31-34) when treated with 2-aminoethanol for 24 hours followed by $KMnO_4$ treatment for the same duration showed open colpus without the intine and protoplasm. However, in few specimens the intine and protoplasm was retained inside the open pollen grains. Surprisingly after 48 and 72 hours comparatively more specimens were observed to possess the intine and protoplasm but most of them lost these contents. This experiments also evidently resulted into degradation of intine and protoplasm in majority of pollen grains.

Text-fig. 10.1.

Variation statistical graphs of the original and secondary altered forms of the investigated palm pollen grains. A = monosulcate pollen grains, B = opened pollen grains with endexine and protoplasm, C = the opened, empty ectexine, D = the intine and protoplasm, without ectexine.
I - variation statistical graphs of the non-experimental, and the experimental material with 2-aminoethanol (24, 48 and 72 hours).
II - variation statistical graphs of the experiments with 2-aminoethanol (24, 48 and 72 hours) + 10 ml $KMnO_4$ (24 hours).
III - variation statistical graphs of the experiments with 2-aminoethanol (24, 48 and 72 hours) + 1 ml merkaptoethanol, and the variation statistical graphs of the pollen grains dissolved with glycerine, 50%.
a = non-experimental, b = length of time 24 hours, c = length of time 48 hours, d = length of time 72 hours, e = 5 ml glycerine 50%, length of time 30 days.

Plate 10.1.

1-22. *Arenga pinnata* (WURMB.) MERR. 1. Experiment No.: T-12-60, 2. T-12-62, 3. T-12-64, 4. T-12-65, 5. T-12-67, 6. T-12-62, 7. T-12-63, 8. T-12-64, 9. T-12-65, 10. T-12-68, 11. T-12-61, 12. T-12-62, 13. T-12-63, 14. T-12-60, 15. T-12-67, 16. T-12-68, 17. T-12-60, 18. T-12-62, 19. T-12-62, 20. T-12-63, 21. T-12-65, 22. T-12-66.


Arenga pinnata
Borassus flabellifer
Caryota urens
I.
II.
III.
%
A
B
C
D
a=0
b=24h
c=48h
d=72h
e=glycerine

Text-fig. 10.1.

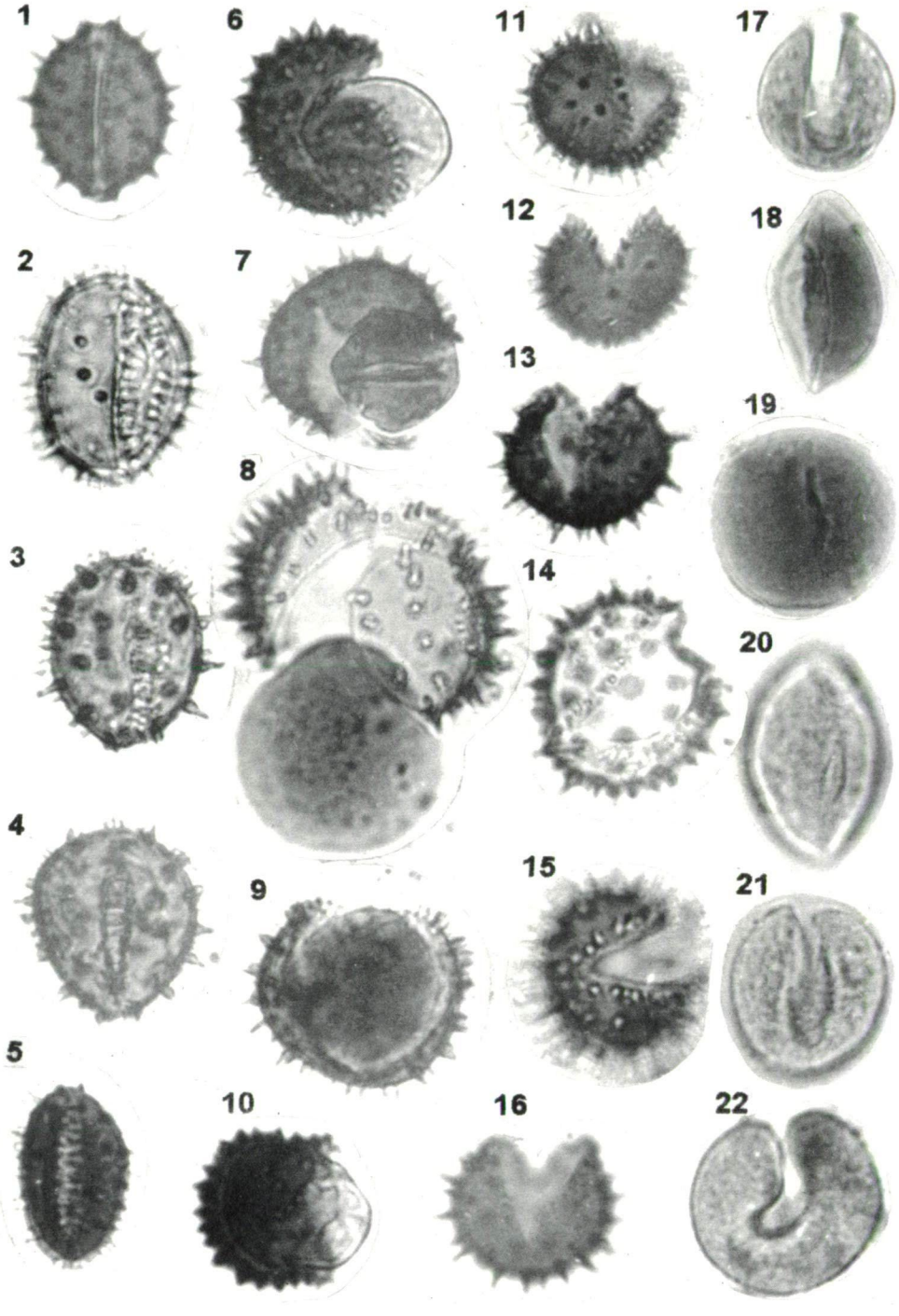


Plate 10.1.

III. In this experiment pollen grains were first treated with 2-aminoethanol for 24, 48 and 72 hours followed by treatment with 1 ml merkaptoethanol for a duration of 24 hours. This experiment showed very interesting results. In response to these treatments pollen grains of *Caryota urens* (Plate 10.4., figs. 22-29, 35, 38) exhibited open colpus and in about half of them intine and protoplasm was degraded. Almost similar result was achieved after the durations of 24, 48 and 72 hours. Pollen grains of *Borassus flabellifer* (Plate 10.3., figs. 5-8, 10-12) when treated for 24 hours showed almost equal number of three types of pollen. One type exhibited the open colpus and possessed intine and protoplasm, whereas, the other type did not possess these contents but had the open colpus. The third type was represented by the intine and protoplasmic contents only indicating that these parts were degraded in one-third pollen grains. After 48 hours only 25% pollen exhibited the presence of intine and protoplasm whereas, after 72 hours no pollen were obsreved to possess these contents. It was noticed that *Borassus flabellifer* pollen were comparatively resistant with regard to the degradation of intine and protoplasm. Almost equal number of *Caryota urens* pollen after being subjected to this experiment for 24 and 48 hours were with or without intine and protoplasm suggesting degradation of these contents in almost 50% of the grains. After 72 hours the degradation was obsrerved to be more effective in these pollen.

Discussion and Conclusions

Statistical analysis of morphological changes brought forth as a consequence to the experiments conducted during present investigations are summarised in Text-fig. 10.1. It is noticed that these experiments did not induce changes in exine ornamentation patterns observable with help of light microscope, instead, the altered features were only with regard to the widening of colpus and exudation of intine and protoplasm.

Arenga pinnata pollen when kept in 50% glycerine for 30 days were found to possess an open colpus but retained the intine and protoplasm within the pollen. Contrary to this, pollen grains of *Borassus flabellifer* and *Caryota urens* (Plate 10.4., fig. 7) after the same treatment, did not retain these elements within the pollen grains. Different kind of changes noticed in three investigated species indicate the selective degradation in exinal and other components of the pollen. Possibly *Arenga pinnata* pollen are more resistant to the degradation causing less expansion in exine preventing the exudation of intine and protoplasm out of the pollen. Susceptibility of the exine is evident in pollen grains of *Borassus flabellifer* and *Caryota urens* where expansion of coplus is more frequent allowing the migration of intine and protoplasm out of the pollen grains.

Plate 10.2.

1-10. *Borassus flabellifer* LINN. 1. Experiment No.: T-12-49, 2. T-12-51, 3. T-12-53, 4. T-12-49, 5. T-12-55, 6. T-12-56, 7,8. T-12-57, 9. T-12-49, 10. T-12-54.

Plate 10.3.

1-12 *Borassus flabellifer* LINN. 1. Experiment No.: T-12-51, 2. T-12-49, 3,4. T-12-53, 5-7. T-12-57, 8. T-12-57, 9. T-12-54, 10,11. T-12-56, 12. T-12-57.

Plate 10.4.

1-38. *Caryota urens* LINN. 1. Experiment No.: T-12-71, 2. T-12-72, 3. T-12-73, 4. T-12-74. 5. T-12-75, 6. T-12-76, 7. T-12-80, 8. T-12-72, 9-11. T-12-73, 12--15. T-12-75, 16-21. T-12-76, 22-27. T-12-78, 28,29. T-12-79, 30. T-12-72, 31,32. T-12-74, 33,34. T-12-75, 35. T-12-79, 36,37. T-12-73, 38. T-12-79.

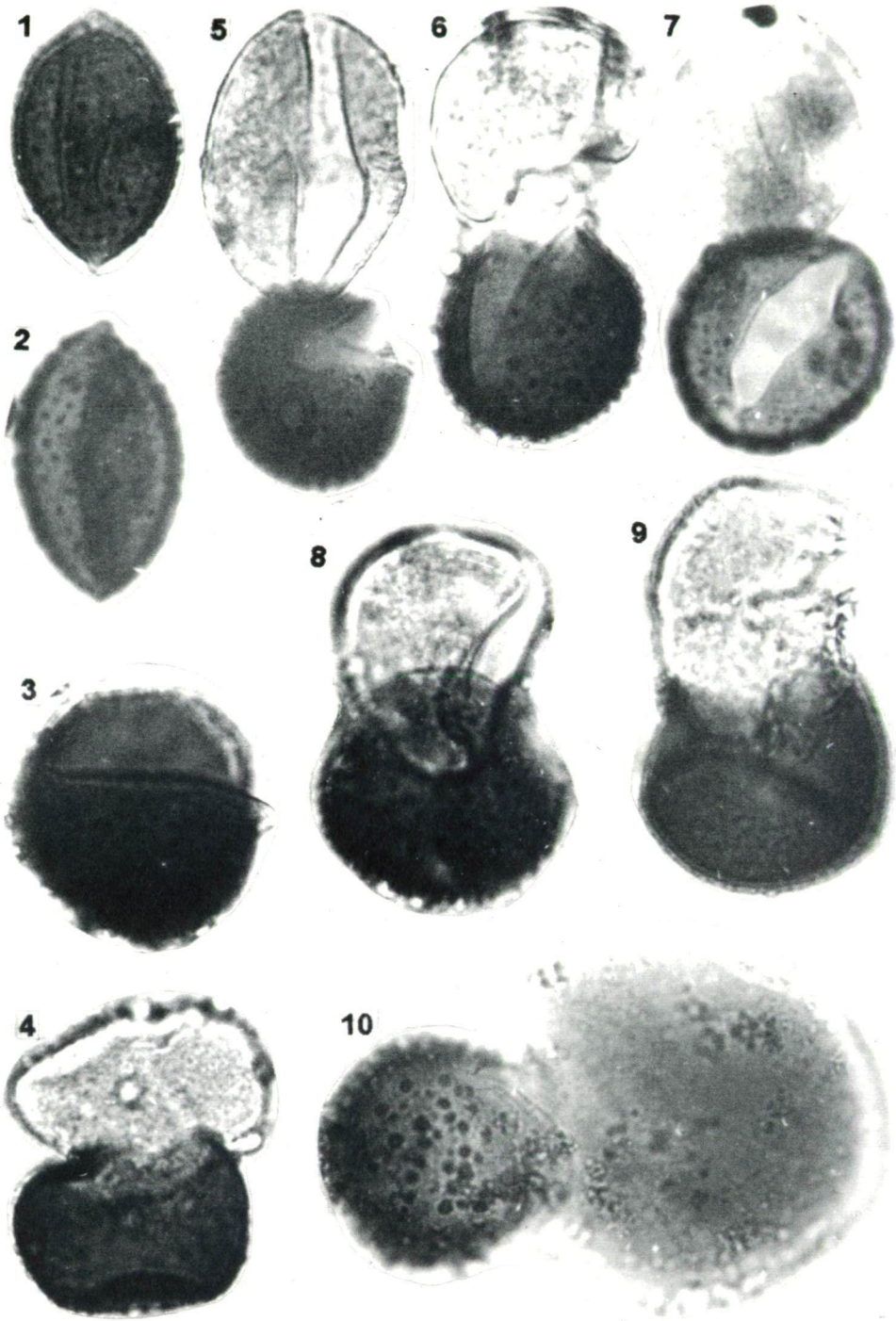


Plate 10.2.

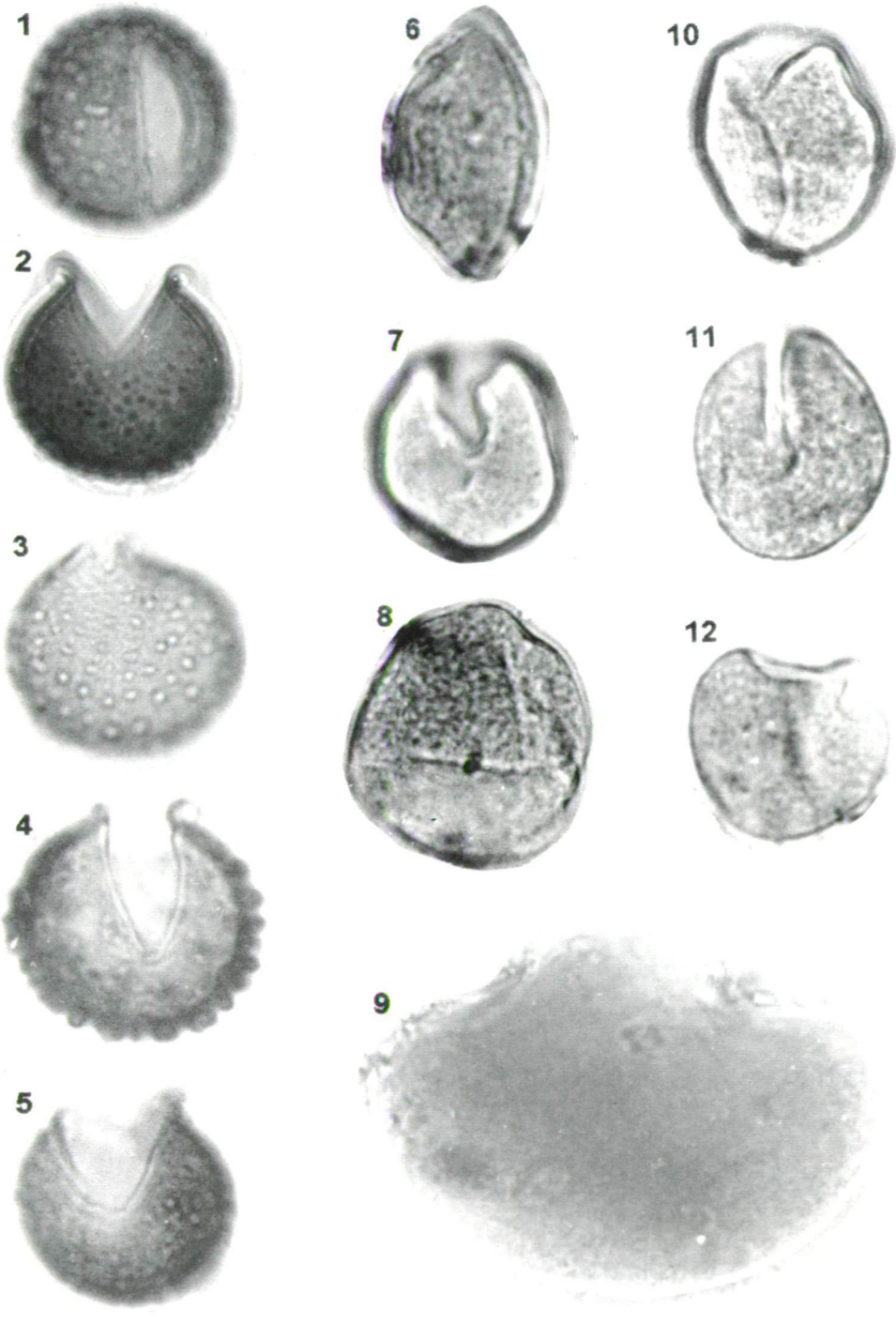


Plate 10.3.

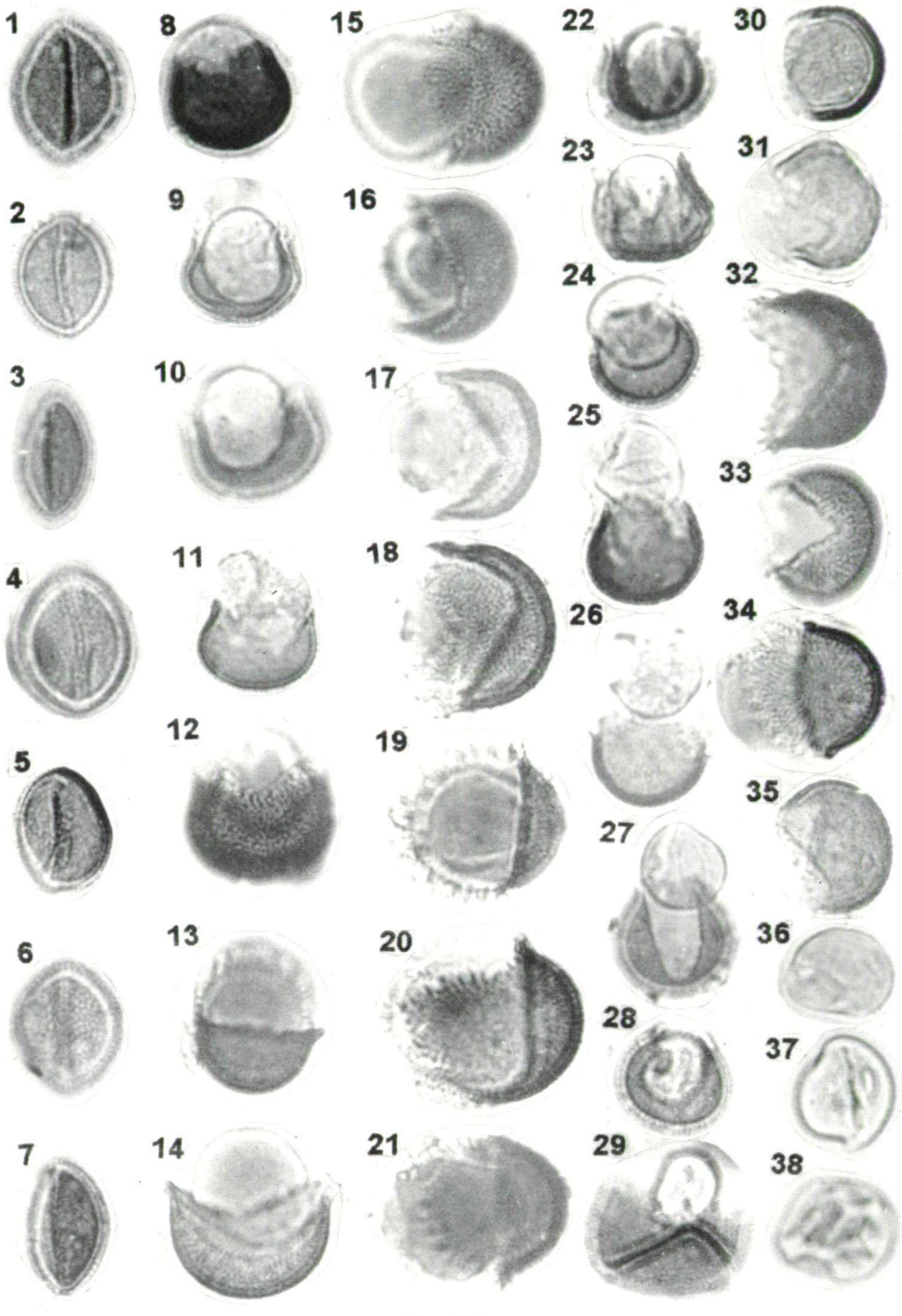

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Plate 10.4.

In response to the 2-aminoethanol treatment pollen grains of *Borassus flabellifer* lost intine and protoplasm particularly after 72 hours. It is indicative of a susceptible nature of exine which because of degradation of its certain part allowed the flow of intine and protoplasm out of the pollen grains. But the situation was not similar in pollen of *Arenga pinnata* and *Caryota urens*. In these species at least in half of the pollen grains these contents were retained within the pollen although the colpus was open.

Similar results were observed when pollen of these species after treatment with 2-aminoethanol were further treated with 1% dil. $KMnO_4$ for 24 hours. Large number of *Arenga pinnata* pollen were observed with open colpus and were without intine and protoplasm. In some specimens these contents were not completely driven out of the pollen (Plate 10.1., figs. 6-8) while in others these were completely separated from the exine but were still housed inside the pollen grains (Plate 10.1., fig. 9). In some specimens the intine and protoplasm were intact but no trace of ectexine was observed. In these specimens interestingly a clear opening of the colpus was noticed (Plate 10.1., figs. 17-22). In these pollen grains possibly selective degradation of ectexine took place as the endexine clearly possessed the aperture.

Selective degradation of ectexine is noticed in at least 20-40% grains as a result of the chemical treatment to *Borassus flabellifer* pollen. Swelling of the inner contents of the pollen from one and half time to almost double in size is the characteristic feature observed in the pollen of *Borassus flabellifer* (Plate 10.2., fig. 11; Plate 10.3. figs. 8, 9). About half of the *Caryota urens* pollen grains when treated with 2-aminoethanol exhibited widely open colpus and separation of endexine along with the protoplasm. Further studies on partially degraded pollen of these species with TEM and SEM is necessary to understand the degradation pattern more clearly.

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References

AMBWANI K. and KUMAR, M. (1991): SEM studies of pollen in *Nypa fruticans* WURMB.- Phytomorphology *41*, 253-255.

DUHOUX, E. (1975): L'aperture dans l'exine et dans l'intine extreme du pollen de *Juniperus chinensis* L., et de *Juniperus communis* L. - Pollen et Spores *17*, 121-201.

EL-GHAZALY, G., ROWLEY, J. and HESSE, M. (1998): Polarity, aperture condition and germination in pollen of *Ephedra* (Gnetales). - Plant Systematic and Evolution *213*, 217-231.

KEDVES, M., KÁROSSY, Á. and BORBOLA, A. (1997): LM investigations of partially dissolved spormorphs I. - Plant Cell Biology and Development (Szeged) *8*, 44-55.

ROWLEY, J.R., and PRIJANTO, B. (1977): Selective degradation of the exine of pollen grains. - Geophytology *7*, 1-23.

SOUTHWORTH, D. (1988): Isolation of exines from gymnosperm pollen. - Amer. J. Bot. *75*, 15-21.

STANLEY, R.G. and LINSKENS, H.F. (1974): Pollen: Biology, Biochemistry, Management. - Springer-Verlag, Berlin.

11. SYMMETRY OPERATIONS ON THE QUASI-PERIODIC BIOPOLYMER STRUCTURES OF THE WALL OF BOTRYOCOCCUS BRAUNII KÜTZ. I.

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Abstract

Using the two dimensional symmetry operations on the biopolymer structures of the partially degraded colonies of *Botryococcus braunii* isolated from Upper Pliocene oil shale from Pula, new data were obtained. Connected regular pentagons (biopolymer A and B) and one in oblique position (biopolymer C) were rotated with fivefold and tenfold primary rotation. The fivefold primary rotation resulted the quasi-crystalloid molecular structure of one globular biopolymer unit. At biopolymer C three globular units were observed in the TEM pictures, the fivefold rotation revealed the complete regular pentagon. A great number of secondary points of symmetry appeared, which may be useful for further symmetry operations.

Key words: Botryococcus braunii fossil, biopolymer symmetry operations.

Introduction

The different kinds of pecularities of the oil producting alga, *Botryococcus braunii* KÜTZ., were pointed out in several papers. During previous times the chemical composition of the wall was separated from the sporopollenin and the terms alganeane, later botryococcene (for the recent) and botryococcane (for the fossil colonies) were introduced, cf. BERKALOFF et al. (1983), KADOURI et al. (1988), ARAUJO et al. (1998). Biopolymer structures which may be modelled with the fullerenes were observed first on the partially degraded and fragmented wall of *Botryococcus* colonies. (KEDVES, ROJIK and VÉR, 1991). The metastable quasi-crystalloid biopolymer skeleton which is also present in the wall of *Botryococcus braunii* was investigated in detail by KEDVES, TÓTH and FARKAS (1993) and KEDVES, TÓTH and VÉR (1995). The quasi-periodic and quasi-equivalent biopolymer symmetry are in contradiction, this is also a peculiarity of the biopolymer organization of the *Botryococcus* colonies. To obtain information concerning the connections of the two kinds of organization, the first attempt were made by KEDVES, TRIPATHI, VÉR, PÁRDUTZ and ROJIK (1998). In this paper it was pointed out, that in spite of some advancement, this problem is not sufficiently resolved.

During our new combined researches, different kinds of partial degradations were carried out and the investigations were made with LM, SEM and TEM method (KEDVES et al., 2000). TEM results of some partially degraded colonies are suitable for symmetry operations. Three peculiar regular pentagons were the subject of the present investigations.

The aim of this paper to obtain fresh informations about the peculiar biopolymer system of this kind of colony.

Materials and Methods

In one previous paper (KEDVES et al. 2000) the first part of the results of the new experimental study of *Botryococcus* colonies were published. The experiment No. AKP-99-7. resulted in suitable biopolymer structures for symmetry operations. Negatives taken with Opton Zeiss instrument (resolution 2-3 Å), were used. The basic two-dimensional rotation methods were used (KEDVES, 1989a, b) with new combinations.

Two complete and one incomplete regular pentagons were the subject of our investigations.

Results

Biopolymer "A" and "B" are connected with one "common" globular biopolymer unit of the regular pentagons. In consequence of the position of the pentagon "C" three globular units were well seen in our picture. Two units are "common" with the pentagon "B". The units C/3 and C/4 were supposed (Plate 11.1.). The primary rotations centre (P) and the rotation axes P-A/X are indicated in this figure.

Biopolymer "A"

C.P.5.A.5.5. (Plate 11.2.) rotation was carried out twice. There are minor differences between the two results. The basic pentagon was reinforced and a light stellate area appeared. At the apices of this light star there are characteristic dark points of symmetry. At the first globular unit of the second fivefold rotation the cyclic molecular cluster was also appeared. The tenfold rotation resulted ten dark points of symmetry, this circle is followed by ten not so characteristic light points of symmetry. Large dark units are around this light sometimes radially elongated light area. Further dark points of symmetry are in the deepest part of the light radiating area its number is in all probability 20. It is a dark border around the light radiating zone composed of several globular points of symmetry.

Biopolymer "B"

C.P.5.A.5.5. (Plate 11.3.) resulted in two regular pentagons composed by not so characteristic points of symmetry. The first point of symmetry of the outer pentagon is in the direction of the rotation axis. The points of symmetry of the inner pentagon are oriented in the middle of the sides of the outer pentagon. A light pentagon represent the rotation area. Around of this area there are characteristic dark points of symmetry. C.P.5.A.5.10. (Plate 11.3.). In one circle ten not so characteristic, more or less triangular points of symmetry, appeared. This circle is surrounded by a light radiating zone. Around this zone there are characteristic dark points of symmetry.

Plate 11.1.

Botryococcus braunii KÜTZ. TEM picture of the biopolymer system of the partially degraded colonies with experiment A.K.P.-99-7A. The biopolymers A, B and C, and its rotation axes are indicated, 2,000.000x.

Plate 11.2.

Rotation pictures of the biopolymer A. The upper two and the lower picture left is 1,000.000, the lower right picture, 2,500.000x.

Plate 11.3.

Five and tenfold rotation pictures of biopolymer B and C: 1,000.000x.

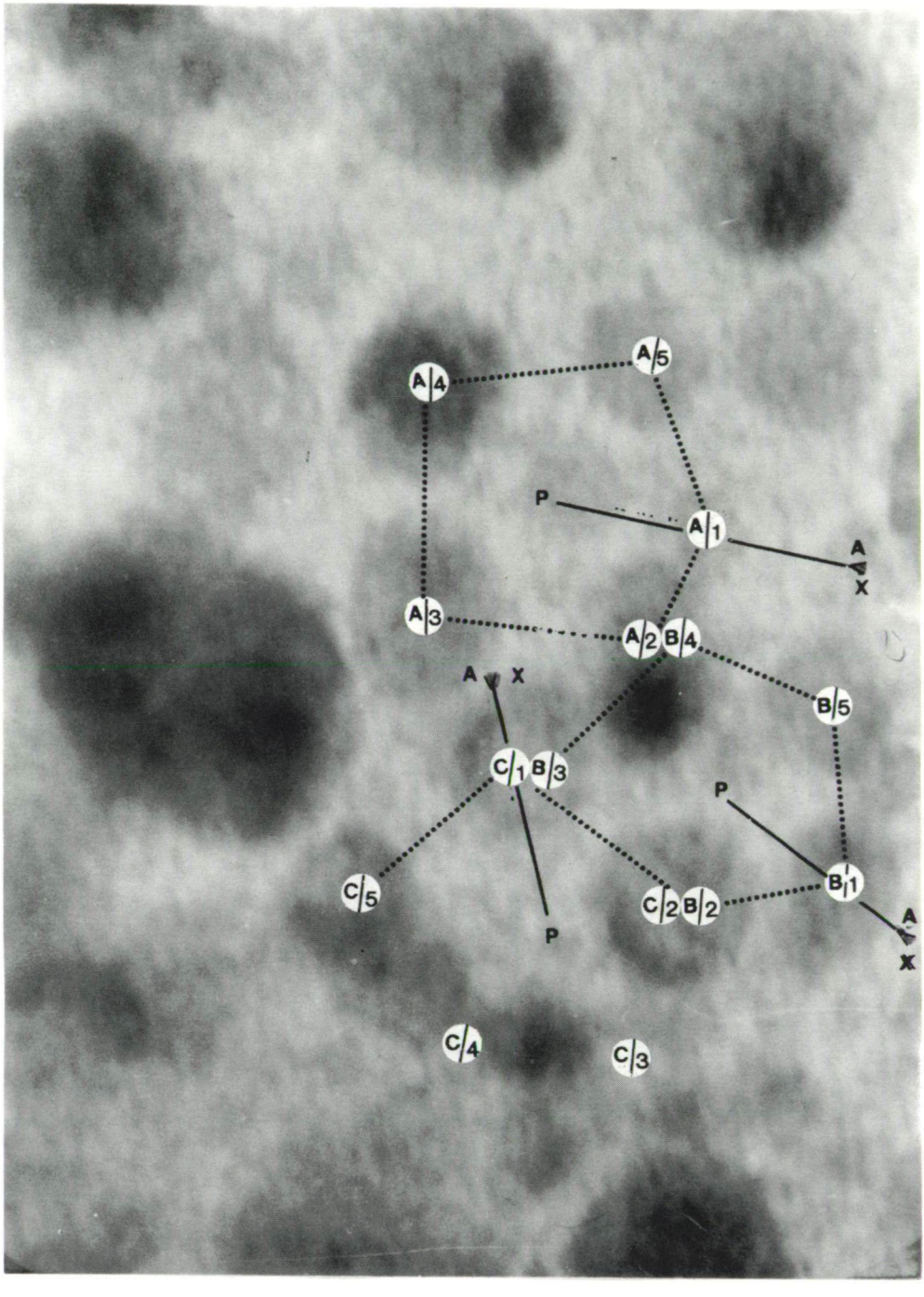
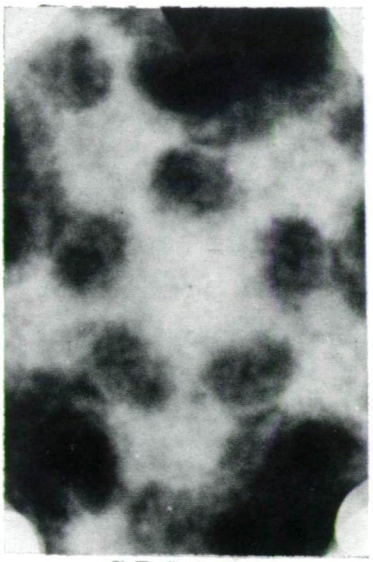


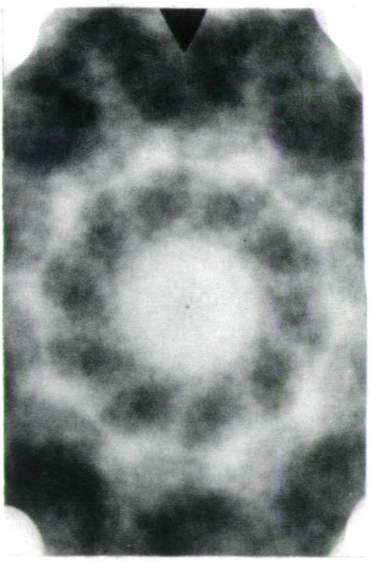
Plate 11.1.

BIOPOLYMER A



C.P.5.A.5.5.

C.P.5.A.5.5.

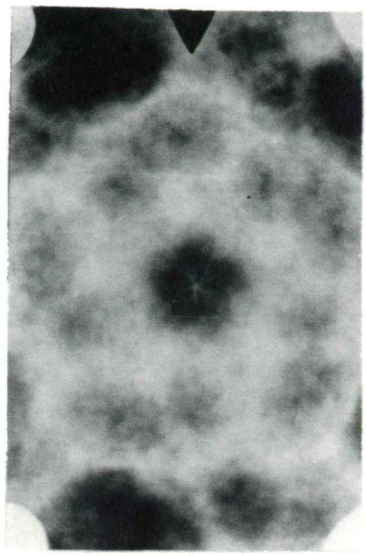


C.P.5.A.5.10.

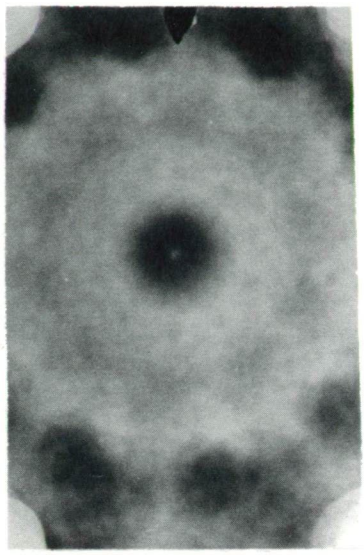
C.P.5.A.5.5.

Plate 11.2.

BIOPOLYMER B

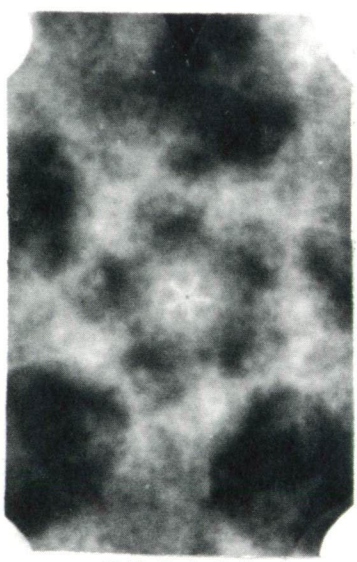


C.P.5.A.5.5.

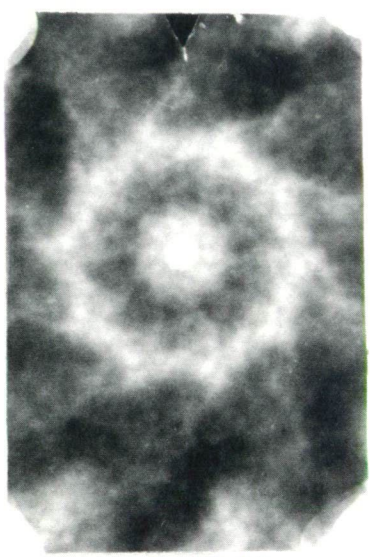


C.P.5.A.5.10.

BIOPOLYMER C



C.P.5.A.5.5.



C.P.5.A.5.10.

Plate 11.3.

Biopolymer "C" (Plate 11.3.)

C.P.5.A.5.5. were made with three globular units of the pentagon two units were expected. The fivefold rotation verified the "absent" two units , but in the centrum of the rotation another small pentagon appeared also. This is surrounded with a light more or less star forming area. The large "original" biopolymer units of the pentagon are in the sides of the light stellate area. C.P.5.A.10. rotation verified the "small pentagon". Ten characteristic dark points of symmetry appeared in a radially oriented triangular zone. A light oblique radiating zone follow the dark points of symmetry. This is surrounded by ten also oblique dark fields composed of small dark globular units.

Discussion and Conclusions

Two regular pentagons were rotated (A, B) with one common unit (A/2, B/4). The presumed pentagon C was originally represented with three globular units (C/1, C/2, C/5). C/1 is common with B/3 respectively C/2 with B/2. C/3 and C/4 was presumed.

1. The repeated rotation revealed that there are differences between the results.
2. Molecular cluster was demonstrated.
3. Fivefold rotation resulted two pentagons in opposite position at biopolymer B.
4. At the tenfold rotation at biopolymer B the points of symmetry of the extra rotation area are characteristic.
5. The pentagon C was represented by three globular units, the fivefold rotation discovered the total regular pentagon. Small pentagon appeared in the middle of this large pentagon. The tenfold rotation picture verified the oblique position of this regular pentagon.

Taking into consideration the interesting results of the secondary rotations of the extra areal points of symmetry further rotations are planned on larger photographic paper to get more informations about the remote points of symmetry from the rotation centrum.

Acknowledgements

The writers are grateful to E. CAULTON (Scottish Centre for Pollen Studies, Edinburgh, Scotland, U.K.) for critically reading the manuscript. This work was supported by Grant OTKA T/9 023208.

References

ARAUJO, C.V., LOUREIRO, M.R.B., BARBANTI, S.M.B., GONÇALVES, F.T.T., MIRANDA, A.C.M.L. and CARDOSO, J.N. (1998): Terrestrial molecular fossils in a typical lacustrine type *Botryococcus*-rich oil shale from Gandarela Basin, Brazil. - Revista Latino Americana de Geoquimica Organica *4*, 25-39.

BERKALOFF, C., CASADEVALL, E. LARGEAU, C., METZGER, P., PERACCA, S. and VIRLET, J. (1983): The resistant polymer of the hydrocarbon-rich alga *Botryococcus braunii*. - Phytochemistry *22*, 389-397.

KADOURI, A., DERENNE, S. LARGEAU, C., CASADEVALL, E. and BERKALOFF, C. (1988): Resistant biopolymer in the outer walls of *Botryococcus braunii*, B race. - Phytochemistry *27*, 551-557.

KEDVES, M. (1989a): Méthode d'étude des biopolymères de la paroi pollinique à structure quasi-cristalloïde. A method of investigation of the quasi-cristalloid structure of pollen wall biopolymers. - Revue de Micropaleontologie *32*, 226-234.

KEDVES, M. (1989b): Quasi-crystalloid biopolymer structures of the sporoderm and its highly organized degrees. - Acta Biol. Szeged. *35*, 59-70.

KEDVES, M., PÁRDUTZ, Á., MADARÁSZ, M., TOMBÁCZ, D., HORVÁTH, A., SZÉCSÉNYI, A., SASHALMI, J., TERBE, ZS., HORVÁTH, ESZTER and KOVÁCS, E. (2000): LM, SEM and TEM investigations on partially degraded *Botryococcus braunii* KÜTZ. colonies from Hungarian Upper Tertiary oil shale I. - Plant Cell Biology and Development (Szeged) *12*, 63-86.

KEDVES, M., ROJIK, I. and VÉR, A. (1991): Biopolymer organization of the partially degraded oil shale with the fragmentation method. - Plant Cell Biology and Development (Szeged) *1*, 28-31.

KEDVES, M., TÓTH, A. and FARKAS, E. (1993): An experimental investigation of the biopolymer organization of both recent and fossil sporoderm. - Grana Suppl. *1*, 40-48.

KEDVES, M., TÓTH, A. and VÉR, A. (1995): Radial fivefold rotation: A new method in the study of the biopolymer organization of the sporoderm. - Plant Cell Biology and Development (Szeged) *6*, 44-59.

KEDVES, M., TRIPATHI, S.K.M., VÉR, A., PÁRDUTZ, Á. and ROJIK, I. (1998): Experimental studies on *Botryococcus* colonies from Hungarian Upper Tertiary oil shale. - Plant Cell Biology and Development (Szeged) *9*, 43-63.

12. C60 FULLERENE/BENZOL SOLUTION AS AN AGENT OF PARTIAL DEGRADATION OF BOTRYOCOCCUS BRAUNII KÜTZ. COLONIES FROM HUNGARIAN ALGINITE

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Short communication

The pecularities in the biopolymer organization of the wall of the *Botryococcus braunii* colonies and the most important establishments were briefly discussed in the previous contribution of this number (KEDVES, SASHALMI and SZÉCSÉNYI, 2002). From the point of view of our new experiments, the presence of the biopolymer structures, which may be modelled with fullerenes, is important. The opportunities in the partial degradation of the fullerene/benzol solution were recognized previously (KEDVES, 1996). Our first experiments are as follows: 10 mg fullerene was soluted in 100 ml benzol, and pure *Botryococcus braunii* KÜTZ. colonies from the Alginite of Várpalota (Transdanubia, Hungary) were the experimental material. 2 mg dried material + 5 ml fullerene/benzol solution, temperature: 30°C, length of time 24 hours, washing with pure benzol, and the dissoluted colonies were dried. Experiment No.: T-12-109. – the dried colonies after experimentation were embedded in Araldite. Experiment No.: T-12-110. – the dried colonies were postfixed in OsO_4 aq. dil. and embedded in Araldite. Experiment No.: T-12-111. – 2 ml merkaptoethanol were added to the dried colonies, and after washing the newly dried material was embedded in Araldite without OsO_4 postfixation. Results. – Experiment T-12-109. (Plate 12.1., figs. 1,2). Dark and light globular units were discovered. The small dark points are of 5-30 Å, the larger light units (holes) 10-70 Å. Sometimes large electron dense globular units are arranged in a regular pentagon. Experiment No.: T-12-110. Different kinds of degradation of the cups were observed (Plate 12.1., fig. 3). In the substance of the cups sometimes linear electron dense structures were observed. In highly magnified pictures there are globular units. Experiment No.: T-12-111. (Plate 12.1., figs. 4,5). Electron dense globular units of 5-60 Å in diameter, and light globular holes of 10-40 Å in diameter appeared after this experiment also. In one part of the partially degraded wall the light holes are arranged in more or less regular pentagon, and hexagons. Two pentagon and two hexagon were observed (Plate 12.1., fig. 5 and schemas A and B). Concerning the hexagons similar electron dense structures were published by MORBELLI and ROWLEY (1996) by confocal study of *Selaginella* megaspore wall. HEMSLEY et al. (1994, 1998) and HEMSLEY (1998) simulated the colloidal sporopollenin in *Selaginella* megaspores by polystyrene latex, cyclohexane and water and nonlinear pattern were demonstrated, between them hexagonal structures also.

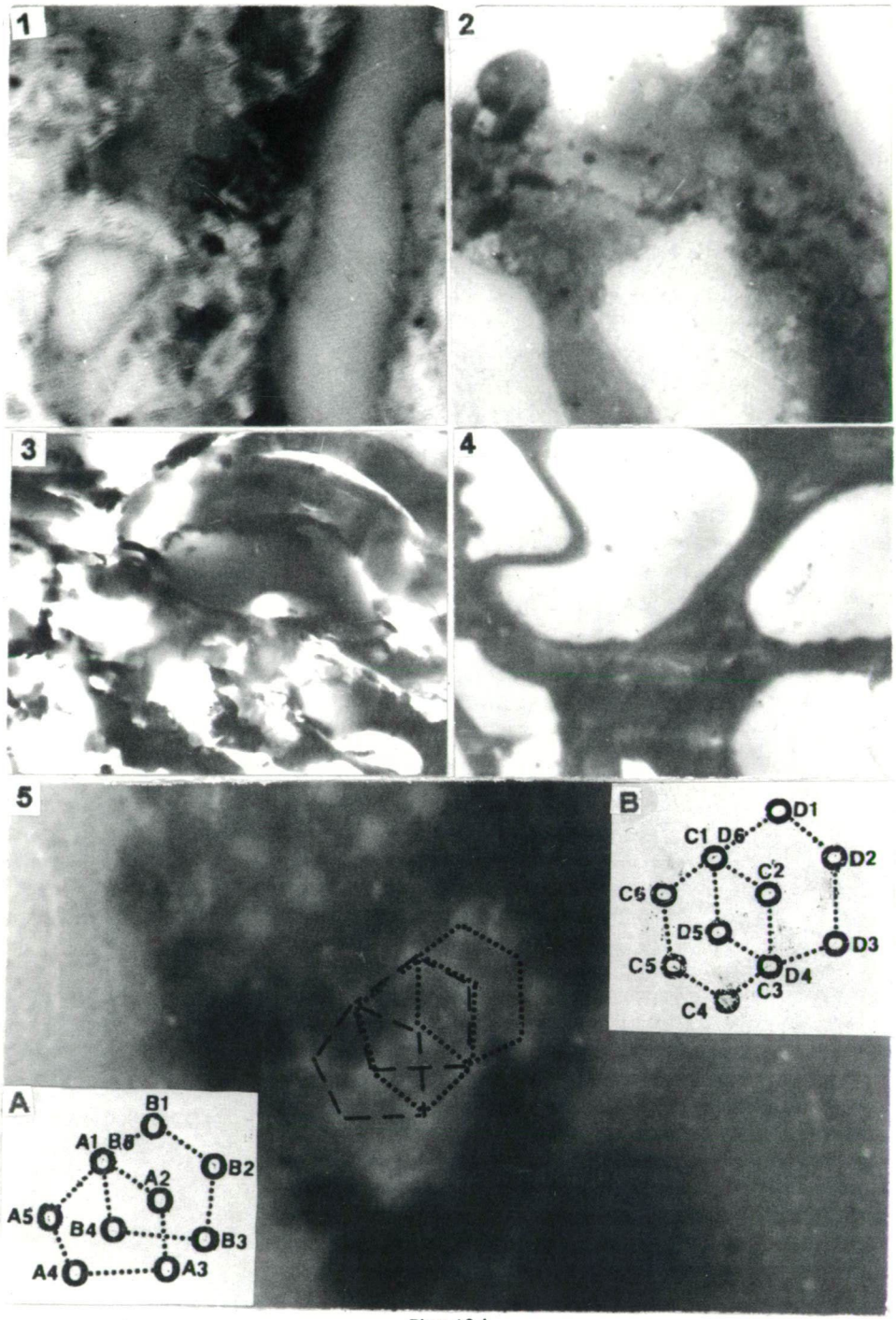
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Plate 12.1.

Plate 12.1

1-5. *Botryococcus braunii* KÜTZ. TEM pictures of the experimentally degraded colonies.
1,2. Experiment No.: T-12-109., 1. Negative No.: 8426, 100.000, 2. Negative No.: 8427, 100.000x.
3. Experiment No.: T-12-110., Negative No.: 8479, 5.000x.
4,5. Experiment No.: T-12-111., 4. Negative No.: 8441, 5.000x, 5. Negative No.: 8434, 250.000x.
A: Schema of the light globular holes forming two pentagons.
B: Schema of two hexagons which are formed by light globular holes.

Finally, we can emphasize, that the basic method seems to be resolved further partial degradation with the fullerene/benzol solution. Based on the first results the highly organized biopolymer network (quasi-crystalloid and quasi-equivalent) may be degraded with this method. But a detailed methodical investigations are necessary for the systematic application of this method.

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The writers are grateful to Prof. Dr. I. KIRICSI (Department of Applied and Environmental Chemistry of the University of Szeged, Hungary), providing us the C60 fullerene and to E. CAULTON (Scottish Centre for pollen Studies, Edinburgh, Scotland, U.K.) for critically reading the manuscript. This work was supported by Grant OTKA T 031725.

References

HEMSLEY, A. R. (1998): Nonlinear Variation in Simulated Complex Pattern Development. – J. theor. Biol. *192*, 73-79.

HEMSLEY, A. R., COLLINSON, M. E., KOVACH, W. L., VINCENT, B. and WILLIAMS, T. (1994): The role of self-assembly in biological systems: evidence from iridescent colloidal sporopollenin in *Selaginella* megaspore walls. – Phil. Trans. R. Soc. Lond. *B, 345*, 163-173.

HEMSLEY, A. R., VINCENT, B., COLLINSON, M. E. and GRIFFITHS, P. (1998): Simulated Self-assembly of Spore Exines. – Annals of Botany *82*, 105-109.

KEDVES, M. (1996): Biopolimer struktúrák szimmetriája. In: Szimmetria-aszimmetria Megközelítések, értelmezések, ed.: BALOGH, T. – MTA, SZAB, Szeged, 7-18.

KEDVES, M., SASHALMI, J. and SZÉCSÉNYI, A. (2002): Symmetry operations on the quasi-periodic biopolymer structures of the wall of *Botryococcus braunii* KÜTZ. I. – Plant Cell Biology and Development (Szeged) *14*, 85-91.

MORBELLI, M. A. and ROWLEY, J. R. (1996): Application of Confocal Microscopy to Exospore Ultrastructure in Megaspores of *Selaginella convoluta* and *S. marginata*. – J. Plant Res. *109*, 37-44.

13. HELICAL BIOPOLYMER ORGANIZATION IN CHANNELS OF THE TECTUM OF PARTIALLY DEGRADED POLLEN GRAINS OF ALNUS GLUTINOSA

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Short communication

Helical biopolymer structure was first described by ROWLEY, DAHL and ROWLEY (1980) from partially degraded ectexine of *Artemisia vulgaris*. Later using the atomic force microscope ROWLEY, FLYNN and TAKAHASHI (1995) described helical biopolymer structures from the pollen exine in *Nuphar*. Investigations with atomic force and scanning tunneling microscopy (WITTBORN, RAO, EL-GHAZALY and ROWLEY, 1996), and scanning tunneling microscopy (WITTBORN, RAO, EL-GHAZALY and ROWLEY, 1998) illustrated helical units. During our TEM investigations of partially degraded exines we observed helical structures as well as quasi-periodic of quasi-equivalent structure, l. c.: KEDVES, BORBOLA, TRIPATHI and MADHAV KUMAR (2000). In our present results, the papers of FLYNN and ROWLEY (1971) and ROWLEY, EL-GHAZALY and ROWLEY (1987) are particularly important with respect to tapetal channels.

During our investigations on allergenic pollen grains interesting structures were observed in the partially degraded ectexine of *Alnus glutinosa* (L.) GAERTN. The TEM pictures taken with an Opton EM-902 instrument (resolution 2-3 Å) resolved well defined globular biopolymer units in a helical arrangement.

The general survey picture (Plate 13.1., fig. 2) illustrates the ultrastructure of the ectexine after partial degradation. The electron dense layer is shown at the surface of the tectum, in the tectum channels, in the surfaces of the elements of the infratectal layer, and the inner surface of the foot layer. In highly magnified micrographs (Plate 13.1., figs. 1,3) tectal channels (20-31 Å in diameter) are illustrated. Evident are electron dense globular granules (3-5 Å) in a helical arrangement (Plate 13.1., figs. 1,3). In lower part of picture 1, the helical organization of the channel of the ectexine is evident. This organization was documented in several publications of ROWLEY.

In conclusion this contribution support how complex is the molecular and biopolymer structure of the pollen wall.

The writers are very grateful to Dr. J.R. ROWLEY (Stockholms Universitet, Botaniska institutionen) for his comments. This work was supported by Grant OTKA T 031715.

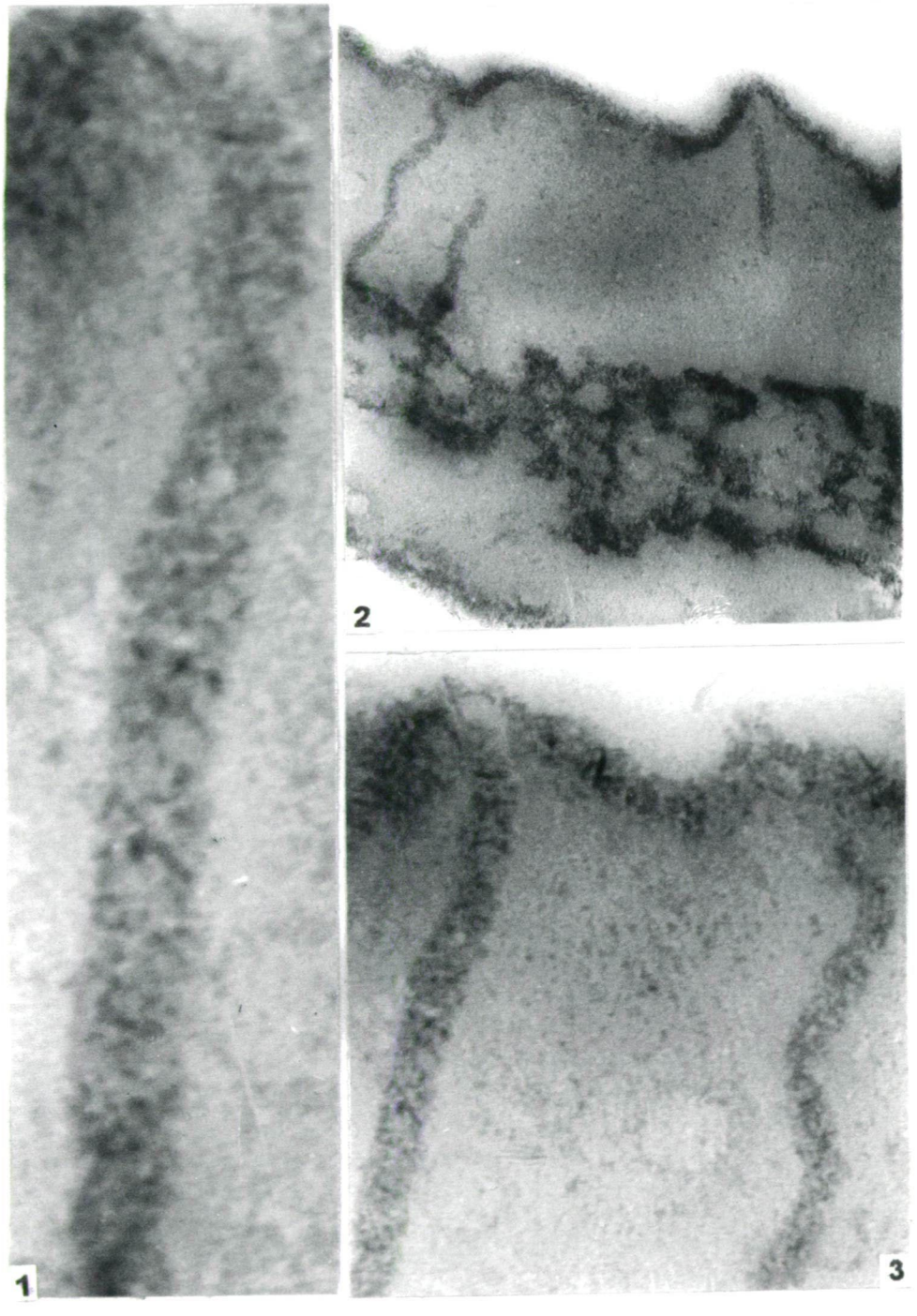


Plate 13.1.

Plate 13.1.

1-3. *Alnus glutinosa* (L.) GAERTN. partially degraded ectexine. Experiment No.: T-12-16.
1. Biolpolymer organization of the tectum channel. Illustrated are the globular electron dense biopolymer units, and the helical organization of the channel. Negative No.: 9714, 500.000x.
2. General survey picture of the partially degraded ectexine. Negative No.: 9712, 100.000x.
3 Detail of the partially degraded tectum channels. Negative No.: 9714, 250.000x.

References

FLYNN, J.J. and ROWLEY, J.R. (1971): Wall microtubules in pollen grains. Zeiss Infor. *76*, 40-45.

KEDVES, M., BORBOLA, A., TRIPATHI, S.K.M. and MADHAV KUMAR (2000): Transmission electron microscopic studies on partially degraded pollen grains of *Phoenix sylvestris* LINN. - Plant Cell Biology and Development (Szeged) *12*, 93-103.

ROWLEY, J.R., DAHL, A.O. and ROWLEY, J.S. (1980): Coiled construction of exinous units in pollen of *Artemisia*. - 38th Ann. Proc. Electron Microscopy Soc. Amer.", San Francisco, California, 1980 G.W. BAILEY (ed.) 252-253.

ROWLEY, J.R., EL-GHAZALY, G. and ROWLEY, J.S. (1987): Microchannels in the pollen grain exine. - Palynology *11*, 1-21.

ROWLEY, J.R., FLYNN, J.J. and TAKAHASHI, M. (1995): Atomic Force Microscope Information on Pollen Exine Substructure in *Nuphar*. - Bot. Acta *108*, 300-308.

WITTBORN, J., RAO, K.V., EL-GHAZALY, G. and ROWLEY, J.R. (1996): Substructure of spore and pollen grain exines in *Lycopodium, Alnus, Betula, Fagus* and *Rhododendron* investigation with Atomic Force and Scanning Tunneling Microscopy. - Grana *35*, 185-198.

WITTBORN, J., RAO, K.V., EL-GHAZALY, G. and ROWLEY, J.R. (1998): Nanoscale Similarities in the Substructure of the Exines of *Fagus* Pollen Grains and *Lycopodium* Spores. - Annals of Botany *82*, 141-145.

14. LIST OF PUBLICATIONS OF THE LABORATORY UNTIL DECEMBER 2001

Compiled by

D. TOMBÁCZ

Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, H-6701, P.O. Box 993, Szeged, Hungary

JELEN, B., BÁLDI-BEKE, M., CIMERMAN, F., ČAR, J., ĆOSOVIĆ, V., DROBNE, K., FODOR, L., KEDVES, M., MÁRTON, E., MONOSTORI, M., SKABERNE, D., TOUMARKINE, M., and ZAGORŠEK, K. (2000): The Eocene in the Dobrna area. In: Eocene in NE Slovenia and NW Croatia, eds.: JELEN, B., ŠIMUNIĆ, A., DROBNE, K., SKABERNE, D., ĆOSOVIĆ, V., AVANIĆ, R., BÁLDI-BEKE, M., CIMERMAN, F., ČAR, J., FODOR, L., KEDVES, M., MÁRTON, E., MONOSTORI, M., PAVLOVEC, R., PLACER, L., ŠIKIĆ, L., TOUMARKINE, M., TURNŠEK, D. and ZAGORŠEK, K., 99-104.

JELEN, B., BÁLDI-BEKE, M., CIMERMAN, F., KEDVES, M. and TOUMARKINE, M. (2000): Biostratigraphy, age and paleoenvironment. In: Eocene in NE Slovenia and NW Croatia, eds.: JELEN, B., ŠIMUNIĆ, A., DROBNE, K., SKABERNE, D., ĆOSOVIĆ, V., AVANIĆ, R., BÁLDI-BEKE, M., CIMERMAN, F., ČAR, J., FODOR, L., KEDVES, M., MÁRTON, E., MONOSTORI, M., PAVLOVEC, R., PLACER, L., ŠIKIĆ, L., TOUMARKINE, M., TURNŠEK, D. and ZAGORŠEK, K., 124-126.

JELEN, N., BÁLDI-BEKE, M., CIMERMAN, F., KEDVES, M., MONOSTORI, M., TOUMARKINE, M. and ZAGORSEK, K. (2000): Biostratigraphy and paleoenvironment. In: Eocene in NE Slovenia and NW Croatia , eds.: JELEN, B., ŠIMUNIĆ, A., DROBNE, K., SKABERNE, D., ĆOSOVIĆ, V., AVANIĆ, R., BÁLDI-BEKE, M., CIMERMAN, F., ČAR, J., FODOR, L., KEDVES, M., MARTON, E., MONOSTORI, M., PAVLOVEC, R., PLACER, L., ŠIKIĆ, L., +TOUMARKINE, M., TURNŠEK, D. and ZAGORŠEK, K., 121-124.

KEDVES, M. (1999): LM and TEM investigations on Hungarian Neogene lignites. - Acta Palaeobot. Suppl. 2, 319-329.

KEDVES, M. (2001a): Upper Cretaceous pollen grains from Egypt VI. - Plant Cell Biology and Development (Szeged) *13*, 19-21.

KEDVES, M. (2001b): Transmission electron microscopy of Mesozoic terrestrial microfossils. - Asociación Paleontológica Argentina, Publicación Especial 7, 113–116.

KEDVES, M., BORBOLA, A. and PRISKIN, K. (2001): LM and TEM investigations on the Upper Cretaceous Ajkaite of Hungary II. - Plant Cell Biology and Development (Szeged) *13*, 22-31.

KEDVES, M. et FREY, K. (2001): Études expérimentales sur quelques grains de pollen des Amentifères. – XVII^{ème} Symposium de l'Association des Palynologues de Langue Française (A.P.L.F.), Arles, 24–26 septembre 2001, Volume des résumés, 52.

KEDVES, M., HORVÁTH, A., MADARÁSZ, M., SASHALMI, J., SZÉCSÉNYI, A. and TOMBÁCZ, D. (2001): High temperature effect on recent pollen grains of *Tilia cordata* MILL. and *Tilia platyphyllos* SCOP. - Plant Cell Biology and Development (Szeged) *13*, 58-61.

KEDVES, M., HORVÁTH, A., TRIPATHI, S.K.M. and MADHAV KUMAR (2001): Symmetry operations on the quasi-crystalloid biopolymer system of the sporopollenin of *Phoenix sylvestris* LINN. from India. - Plant Cell Biology and Development (Szeged) *13*, 76-86.

KEDVES, M. and MADARÁSZ, M. (2001a): To the Tenth Anniversary of the Cell Biological and Evolutionary Micropaleontological Laboratory. - Plant Cell Biology and Development (Szeged) *13*, 6-10.

KEDVES, M. and MADARÁSZ, M. (2001b): Experimental studies on the pollen grains of Elaeagnus angustifolia L. - Plant Cell Biology and Development (Szeged) *13*, 71-75.

KEDVES, M. and PÁRDUTZ, Á. (2001): Ultrastructure of Partially Degraded Hungarian Lignites. - Taiwania *46*, 135–144.

KEDVES, M., PÁRDUTZ, Á. and MADARÁSZ, M. (1999-2000): A parlagfű virágporszemein elért eddigi kísérletes ultrastruktúra vizsgálataink eredményei. - Botanikai Közlemények *86-87*, 242.

KEDVES, M., PÁRDUTZ, Á. and MADARÁSZ, M. (2001a): Transmission electron microscopy of the partially degraded pollen grains from the Thanetian layers of Menat (France) II. - Plant Cell Biology and Development (Szeged) *13*, 32-39.

KEDVES, M., PÁRDUTZ, Á. and MADARÁSZ, M. (2001b): Transmission electron microscopy of partially degraded teliospores of *Ustilago maydis* (DE CANDOLLE) CORDA. - Palinología: Diversidad y Aplicaciones eds.: FOMBELLA BLANCO, M.A., FERNÁNDEZ GONZÁLEZ, D. and VALENCIA BARRERA, R.M., 341-348.

KEDVES, M., PÁRDUTZ, Á., MADARÁSZ, M., PRISKIN, K., SZÉCSÉNYI, A., SASHALMI, J. and TOMBÁCZ, D. (2001): LM and TEM investigations on partially degraded pollen grains of *Alnus glutinosa* (L.) GAERTN. - Plant Cell Biology and Development (Szeged) *13*, 62-70.

KEDVES, M., PÁRDUTZ, Á., PRISKIN, K., SZÉCSÉNYI, A., SASHALMI, J., TOMBÁCZ, D. and BORBOLA, A. (2001): LM, SEM and TEM investigations on partially degraded *Botryococcus braunii* Kütz. colonies from Hungarian Upper Tertiary oil shale II. - Plant Cell Biology and Development (Szeged) *13*, 40-52.

KEDVES, M., PÁRDUTZ, Á., SZÉCSÉNYI. A., PRISKIN, K., MADARÁSZ, M., TOMBÁCZ, D., SASHALMI, J. and HORVÁTH, A. (2001): LM and SEM investigations on partially dissolved allergen pollen grains I. - Plant Cell Biology and Development (Szeged) *13*, 53-57.

KEDVES, M., PÁRDUTZ, Á., TERBE, Zs. et HORVÁTH, E. (2001): Microscopie électronique à transmission de l'exine partiellement dégradées de grains de pollen de quelques Cycadales. - Plant Cell Biology and Development (Szeged) *13*, 87-95.

KEDVES, M., PRISKIN, K., TRIPATHI, S.K.M. and MADHAV KUMAR (2001): Experimental LM studies on recent palm pollen grains. - Abstracts Congress on Tropical and Subtropical Palynology (America-Africa) February 10-15/2001, 32.

SRIVASTAVA, S.C. and BANERJI, J. (2001): Pentoxylon plant: A reconstruction and interpretation. - Plant Cell Biology and Development (Szeged) *13*, 11-18.

SZÉCSÉNYI, A. (2001): Chronicle. - Plant Cell Biology and Development (Szeged) *13*, 98-103.

TOMBÁCZ, D. (2001): List of publications of the Laboratory until December 2000. - Plant Cell Biology and Development (Szeged) *13*, 96-97.

Chronicle

compiled by

A. SZÉCSÉNYI

Visiting scientists

Dr. S.K.M. TRIPATHI, Scientist 'D', Birbal Sahni Institute of Palaeobotany, Lucknow, India worked in our Laboratory from 19th July to 29th September. This visit was under the Exchange of Scientists Programme between Indian National Science Academy and the Hungarian Academy of Sciences, Budapest. During his stay we worked on partially degraded pollen grains of *Cycas rumphii* MIQ. and on the ultrastructure of partially degraded cuticles of the same species. In a preliminary report quasi-crystalloid biopolymer structure was established for the first time from the experimentally degraded cuticle.Two papers incorporating these results will be published in the next number of Plant Cell Biology and Development. Further combined (SEM, TEM) investigations on the partially degraded cuticles of *Cycas rumphii* MIQ. are in progress.

Prof. Dr. Funda AKGÜN (Izmir, Turkey) visited the Laboratory with her husband from 23rd to 27th July. Fruitful discussions on Tertiary palynomorphs from Turkey were made and she got acquinted with the research and teaching programs of the Laboratory (Photo 1).

Photo 1. – Dr. S. K. M. TRIPATHI, Prof. D. Funda AKGÜN and Prof. Dr. M. KEDVES in the office of the Laboratory.

Eric CAULTON (Director of the Scottish Centre for Pollen Studies) and his wife visited Szeged from 20th - 22nd August. On 21st August at the occasion of 11th Anniversary of the Laboratory he was awarded with the Commemorative Medal of the Laboratory.

Dr. M.T. FERNANDEZ MARRÓN (Consejo Superior de Investigaciones Cientificas, Universidad Complutense de Madrid, España) visited the Laboratory on the 31st August. She was awarded with the Commemorative Medal of the Laboratory.

International Laboratory activities

13 - 28 January, 2001, Lucknow, Uttar Pradesh, India.

Under INSA-Hungarian Academy of Sciences Exchange of Scientists Programme Prof. Dr. M. KEDVES visited India from 14-27 January, 2001. In New Delhi he was received by Mr. A.K. JAIN, Asstt. Executive Secretary of the Indian National Science Academy. In Lucknow he was received by Dr. Suresh C. BAJPAI, Registrar, Dr. S.K.M. TRIPATHI, Scientist 'D' and Dr. MADHAV KUMAR, Scientist 'C'. Fruitful discussion about the scientific relationships were made with Prof. Dr. A.K. SINHA, Director, Birbal Sahni Institute. With Dr. RAKESH SAXENA discussions were continueted about the opportunities of new joint researches.

During this visit a manuscript - Experimental LM studies on recent palm pollen grains, authored by KEDVES, M., PRISKIN, K., TRIPATHI, S.K.M. and MADHAV KUMAR, was finalized. This paper has been published in the present number of Plant Cell Biology and Development.

25 January in the afternoon Dr. S. C. SRIVASTAVA was awarded with the Millenium Medal of the Laboratory. An exclusive reception was held at this occasion. Dr. S.K.M. TRIPATHI was the chairman of this meeting. Prof. Dr. M. KEDVES reviewed the history of the connections between the Laboratory and the Birbal Sahni Institute of Palaeobotany, which started at the 2nd European Palaeobotanical Conference in Madrid, in 1989. In Madrid Dr. S.C. SRIVASTAVA invited Prof. Dr. M. KEDVES to deliver an Inaugural Lecture for "Savitri Sahni Smarak Lecture Series". Following it, commemorative meeting was held in the Banquet Hall of Hotel Clarks Avadh, Lucknow on 19th September, 1990 and joint researches, publications and regular exchange visits started. Dr. S.C. SRIVASTAVA recalled his pleasant souvenir of Szeged. Finally both sides pointed out the advantages of scientific connections (Photo 2.)

8-16 February, 2001, Havanna, Cuba, Congress on Tropical and Subtropical Palynology (America-Africa). Organization: Pr. Dr. Pedro PEREZ, Instituto de Ecologia y Sistematica; Dr. Sonia MACHADO RODRIGUEZ, Instituto de Ecologia y Sistematica; Dr. Raquel CARRERAS, Centro National de Museologia. Secretariat: Dr. Sonia MACHADO RODRIGUEZ. Scientific Board: Permanent Members: Pedro PEREZ, Raquel CARRERAS, Sonia MACHADO RODRIGUEZ; Paleopalynology: Henry HOOGHIEMSTRA (The Netherlands), Emile ROCHE (Belgium), Pedro PEREZ (Cuba); Pollen Morphology: Maruxa SUAREZ-CERVERA (Spain), Annick LE THOMAS (France), Miklós KEDVES (Hungary), Sonia MACHADO RODRIGUEZ (Cuba); Aeropalynology: Delia FERNANDEZ-GONZALES (Spain), F.T.M. SPIEKSMA (The Netherlands), Ofelia GONZALES (Cuba); Melissopalynology: Irene LA SERNA RAMOS (Spain), D. LOBREAU-CALLEN (France), Lazara SOTOLONGO MOLINA (Cuba).

Photo 2. – Dr. S. C. SRIVASTAVA, Prof. Dr. M. KEDVES, Dr. S. K. M. TRIPATHI, Dr. B. N. JANA, Dr. Rakesh SAXENA, Dr. Neeru PRAKASH and Mrs. Rita CHATERJEE in the office of Dr. S. C. SRIVASTAVA

Photo 3. – Prof. Dr. M. KEDVES, Prof. Dr. T.-C. HUANG and Prof. Dr. S. NILSSON before the Hotel Comodoror, Havanna, Cuba.

Photo 4. – Prof. Dr. M. KEDVES, Mrs. L.-C. HUANG and Prof. Dr. T.-C. HUANG before the Hotel Comodoro, Havanna, Cuba

On the 12 February M. KEDVES delivered the following paper:

Experimental LM studies on recent palm pollen grains, authors: M. KEDVES, K. PRISKIN, S.K.M. TRIPATHI and MADHAV KUMAR.

During this conference several fruitful discussions were made with several colleagues, in particular with Prof. Dr. T.-C. HUANG (Photos 3, 4) and Prof. Dr. S. NILSSON (Photo 3).

24-26 September, 2001, Arles, France, XVIIème Symposium APLF.

Organization: Valérie ANDREU-PONEL, IMEP-Université d'Aix-Marseille; Rachid CHEDDADI, IMEP-Univerité d'Aix-Marseille; J.-Louis DE BEAULIEU, IMEP-Université d'Aix-Marseille; Dominique JOLLY, ISEM-Université de Montpellier; Annie VINCENS, CEREGE-Université d'Aix-Marseille. On the 26 September M. KEDVES delivered the following paper: KEDVES, M. et FREY, K.: Études expérimentales sur quelques grains de pollen des Amentifères.

Hungarian scientific activities

On the 2nd January, 2001 appeared the 13th number of Plant Cell Biology and Development.

5th April 2001. At the meeting of the Geonomical Commission of the Hungarian Academy of Sciences the monograph of Prof. Dr. E. SZÁDECKY-KARDOSS was discussed. President: Dr. E. DUDICH. The following speakers reviewed several chapters of this monograph: SZ. BÉRCZI, L. CSEREPES, Z. DITRÓI PUSKÁS, I. KUBOVICS, E. DUDICH and M. KEDVES.

12th April, 2001. A peculiar meeting was organized under the presidenship of Prof. Dr. I. KUBOVICS on the problem of the Supernova Theory (Szupernova Szimpózium) with the following speakers: B. LUKÁCS, L. PATKÓS, B. BALÁZS, CS. DETRE, GY. DON, M. KEDVES, GY. SZÖŐR.

22th May, 2001. The Geological Section of the Hungarian Academy of Sciences organized a commemorative meeting at the occasion of the 120th birth anniversary of Prof. Dr. K. OLTAY. The president of the meeting was Prof. Dr. J. ÁDÁM, member of the Hungarian Academy of Sciences. Speaker: Prof. Dr. K. HORVÁTH.

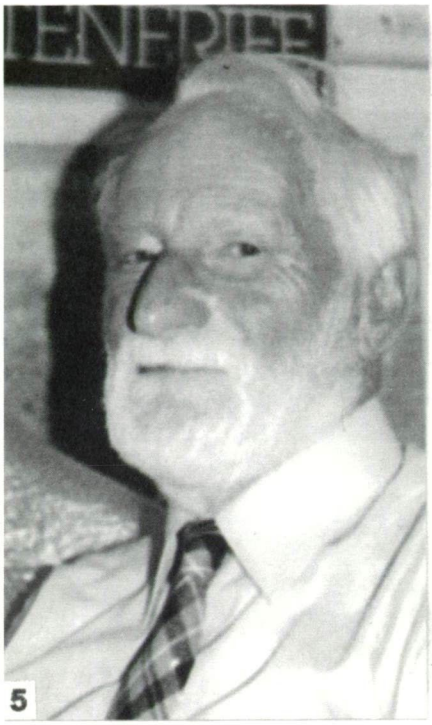
6th June,2001. At the occasion of the 70th birthday of Prof. Dr. M. JÁRAI-KOMLÓDI, the Botanical and the Naturprotective and Conservationbiological Commission of the Hungarian Academy of Sciences and the Ecological Section of the Hungarian Biological Society a festive meeting was held at Hungarian Academy of Sciences. Organization: Prof. Dr. A. BORHIDI member of the Hungarian Academy of Sciences, Prof. Dr. G. FEKETE, member of the Hungarian Academy of Sciences, K. VIRÁG and A. KUN. Speakers: G. FEKETE, A. BORHIDI, K. NÉKÁM, A. HORÁNSZKY, R. MÁTHÉ, and T. SEREGÉLYES. The Laboratory was represented by Prof. Dr. M. KEDVES.

On the meeting of the Botanical Section of the Hungarian Biological Society M. KEDVES delivered the following lecture: C60 fullerén/benzol oldat jelentősége növényi eredetű biopolymer rendszerek megismerésében. Importance of the C60 fullerene/benzol solution in the recognition of the biopolymer systems of plant origin.

Laboratory meetings and news

12.01.2001. – Preparation for the visit to Birbal Sahni Institute of Palaeobotany and for the Congress on Tropical and Subtropical Palynology, Havanna, Cuba.

24.02.2001. – Reports on the visit to Birbal Sahni Institute of Palaeobotany of M. KEDVES and from the Congress on Tropical and Subtropical Palynology held in Havanna, Cuba. Other businesses. Speaker: M. KEDVES.



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31.03.2001. – International joint researches and programs. The state of the Laboratory publications. Speaker: M. Kedves.

02.04.2001. – Miss. K. FREY left the Laboratory. The new laboratory assistant Miss. B. VARGA started to work.

28.04.2001. – The research programs of the Laboratory for the summer, publications of the Laboratory. Speaker: M. KEDVES.

18.05.2001. – Discussion on the diplom thesis of A. BORBOLA was held in the Department of Botany of the University of Szeged. She got top mark. She got a job in the Department of Genetics of the Biological Research Center of the Hungarian Academy of Sciences.

26.05.2001. – The present day state of the research programs of the Laboratory. 14th and 15th number of the Plant Cell Biology and Development. Speaker: M. KEDVES.

21.08.2001. – At 4 pm an exclusive recepcion took place in the Laboratory. Eric CAULTON was awarded with the Commemorative Medal of the Laboratory (Photo 5).

Dr. S.K.M. TRIPATHI was awarded with the Millennium Medal of the Laboratory (Photo 8).

Participants: E. CAULTON, H. CAULTON (Photo 6), S.K.M. TRIPATHI, M. KEDVES, É. SIPOS-KEDVES, A. BORBOLA, ZS. IMRE, K. PRISKIN, J. SASHALMI, A. SZÉCSÉNYI, D. TOMBÁCZ, B. VARGA (Photo 7).

31.08.2001. – Dr. M.T. FERNÁNDEZ MARRÓN (Madrid, Spain) visited the Laboratory. She was awarded with the Commemorative Medal of the Laboratory. She could not attend the 11th meeting, so another exlusive reception was held in the Laboratory at this occasion. Participants: M. T. FERNÁNDEZ MARRÓN, S.KM. TRIPATHI, M. KEDVES, É. SIPOS-KEDVES, A. BORBOLA, ZS. IMRE, J. SASHALMI,, A. TÓTH, B. VARGA (Photo 9, 10).

29.09.2001. – Report from the summer times and from the XVIIth Symposium of A.P.L.F. held in Arles. The financial situation of the Laboratory in the present day conditions. Problems and prospects. Speaker: M. KEDVES.

27.10.2001. – The present day state of the next number of Plant Cell Biology and Development. Programs for the international joint research programs, and participations on the scientific meetings of 2002. Speaker: M. KEDVES.

24.11.2001 – Preparation for the 15th number of Plant Cell Biology and Development, and first discussion for the Laboratory awards for the 2002. year. Speaker: M. KEDVES.

08.12.2001. – Results and problems of the year 2001. Programs for the New Year. State of the joint research programs and contributions with the colleagues from Birbal Sahni Institute of Palaeobotany, Lucknow, India.Speaker: M. KEDVES.

Teaching program of the Laboratory

In the year of 2001 the following lectures were delivered:

1. Applied Palynology, 1+2. 2. Biopolymer organization and symmetry, 1+2. 3. Theory of Evolution and Natural Philosophy, 1+0. 4. Introduction to the prequaternary Palynology, 1+2. 5. Theory of the Supernova, 1+0. 6. Basic Palynology, 2+2. 7. Quasicrystalloid biopolymer structures, 1+2

Photo 5. – E. CAULTON, Director, photo 6. – H. CAULTON, photo 7. – Staff of the Laboratory, from left to right: K. PRISKIN, B. VARGA, Prof. Dr. M. KEDVES, A. BORBOLA, A. SZÉCSÉNYI, ZS. IMRE and D. TOMBÁCZ. Pictures were taken at the exclusive reception on the 21st August by Dr. É. Sipos-Kedves.

Photo 8. – Dr. S. K. M. TRIPATHI, picture taken by Dr. É. SIPOS-KEDVES at the exclusive reception on the 21st August, photo 9. – Dr. M. T. FERNÁNDEZ MARRÓN, photo 10. – Participants at the meeting on 31st August, pictures taken by Dr. É. SIPOS-KEDVES. From left to right: Dr. S. K. M. TRIPATHI, Prof. Dr. M. KEDVES, J. SASHALMI, Dr. M. T. FERNÁNDEZ MARRÓN, B. VARGA and ZS. IMRE.

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